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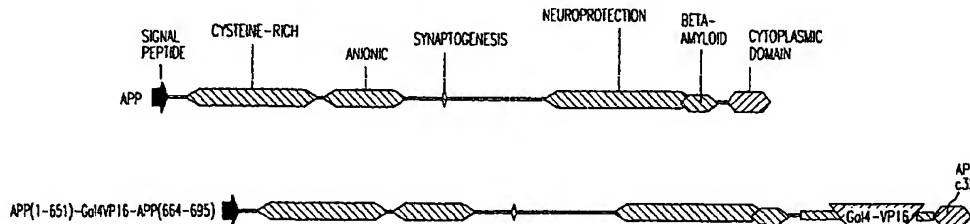
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(54) Title: ASSAYS TO MONITOR AMYLOID PRECURSOR PROTEIN PROCESSING



(57) Abstract: The present invention provides DNA constructs, genetically engineered host cells, and methods for identifying inhibitors of amyloid precursor protein (APP) processing. The methods provide for the convenient identification, in a single assay, of inhibitors of  $\beta$ -secretase and  $\gamma$ -secretase as well as other forms of APP processing. The methods rely on fusion proteins of APP and transcription factors in which APP processing releases the transcription factors, allowing the transcription factors to activate transcription of a reporter gene. Inhibitors are identified as substances that block or diminish transcription factor release from the fusion protein, thereby causing a diminution of reporter gene readout.

WO 03/072041 A2

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TITLE OF THE INVENTION  
ASSAYS TO MONITOR AMYLOID PRECURSOR PROTEIN PROCESSING

CROSS-REFERENCE TO RELATED APPLICATIONS

5                   This application claims the benefit of U.S. Provisional Application No. 60/360,274, filed February 27, 2002, the contents of which are incorporated herein by reference in their entirety.

STATEMENT REGARDING FEDERALLY-SPONSORED R&D

10                   Not applicable.

REFERENCE TO MICROFICHE APPENDIX

Not applicable.

15   FIELD OF THE INVENTION

                  The present invention is directed to the field of Alzheimer's disease. In particular, the present invention provides novel methods of identifying substances that are specific inhibitors of various steps in the processing of amyloid precursor protein.

20

BACKGROUND OF THE INVENTION

                  Alzheimer's disease is a common, chronic neurodegenerative disease, characterized by a progressive loss of memory and sometimes severe behavioral abnormalities, as well as an impairment of other cognitive functions that often leads to dementia and death. It ranks as the fourth leading cause of death in industrialized societies after heart disease, cancer, and stroke. The incidence of Alzheimer's disease is high, with an estimated 2.5 to 4 million patients affected in the United States and perhaps 17 to 25 million worldwide. Moreover, the number of sufferers is expected to grow as the population ages.

25

30                   A characteristic feature of Alzheimer's disease is the presence of large numbers of insoluble deposits, known as amyloid plaques, in the brains of those affected. Autopsies have shown that amyloid plaques are found in the brains of virtually all Alzheimer's patients and that the degree of amyloid plaque deposition correlates with the degree of dementia (Cummings & Cotman, 1995, Lancet

326:1524-1587). While some opinion holds that amyloid plaques are a late stage by-product of the disease process, the consensus view is that amyloid plaques are more likely to be intimately, and perhaps causally, involved in Alzheimer's disease.

A variety of experimental evidence supports this view. For example,  
 5 A $\beta$ , a primary component of amyloid plaques, is toxic to neurons in culture and transgenic mice that overproduce A $\beta$  in their brains show significant deposition of A $\beta$  into amyloid plaques as well as significant neuronal toxicity (Yankner, 1990, *Science* 250:279-282; Mattson et al., 1992, *J. Neurosci.* 12:379-389; Games et al., 1995, *Nature* 373:523-527; LaFerla et al., 1995, *Nature Genetics* 9:21-29). Mutations in the  
 10 APP gene, leading to increased A $\beta$  production, have been linked to heritable forms of Alzheimer's disease (Goate et al., 1991, *Nature* 349:704-706; Chartier-Harlin et al., 1991, *Nature* 353:844-846; Murrel et al., 1991, *Science* 254:97-99; Mullan et al., 1992, *Nature Genetics* 1:345-347). Presenilin-1 (PS1) and presenilin-2 (PS2) related familial early-onset Alzheimer's disease (FAD) shows disproportionately increased  
 15 production of A $\beta$ 1-42, the 42 amino acid isoform of A $\beta$ , as opposed to A $\beta$ 1-40, the 40 amino acid isoform (Scheuner et al, 1996, *Nature Medicine* 2:864-870). The longer isoform of A $\beta$  is more prone to aggregation than the shorter isoform (Jarrett et al, 1993, *Biochemistry* 32:4693-4697). Injection of the insoluble, fibrillar form of A $\beta$  into monkey brains results in the development of pathology (neuronal destruction, tau  
 20 phosphorylation, microglial proliferation) that closely mimics Alzheimer's disease in humans (Geula et al., 1998, *Nature Medicine* 4:827-831). See Selkoe, 1994, *J. Neuropathol. Exp. Neurol.* 53:438-447 for a review of the evidence that amyloid plaques have a central role in Alzheimer's disease.

A $\beta$ , a 39-43 amino acid peptide derived by proteolytic cleavage of the  
 25 amyloid precursor protein (APP), is the major component of amyloid plaques (Glenner & Wong, 1984, *Biochem. Biophys. Res. Comm.* 120:885-890). APP is actually a family of polypeptides produced by alternative splicing from a single gene. Major forms of APP are known as APP695, APP751, and APP770, with the subscripts referring to the number of amino acids in each splice variant (Ponte et al.,  
 30 1988, *Nature* 331:525-527; Tanzi et al., 1988, *Nature* 331:528-530; Kitaguchi et al., 1988, *Nature* 331:530-532). APP is membrane bound and undergoes proteolytic cleavage by at least two pathways. In one pathway, cleavage by an enzyme known as  $\alpha$ -secretase occurs while APP is still in the trans-Golgi secretory compartment (Kuentzel et al., 1993, *Biochem. J.* 295:367-378). This cleavage by  $\alpha$ -secretase

occurs within the A $\beta$  portion of APP, thus precluding the formation of A $\beta$ . In another proteolytic pathway, cleavage of the Met596-Asp597 bond (numbered according to the 695 amino acid protein) by an enzyme known as  $\beta$ -secretase occurs. This cleavage by  $\beta$ -secretase generates the N-terminus of A $\beta$ . The C-terminus is formed  
5 by cleavage by a second enzyme known as  $\gamma$ -secretase. The C-terminus is actually a heterogeneous collection of cleavage sites rather than a single site since  $\gamma$ -secretase activity occurs over a short stretch of APP amino acids rather than at a single peptide bond. Peptides of 40 or 42 amino acids in length (A $\beta$ 1-40 and A $\beta$ 1-42, respectively) predominate among the C-termini generated by  $\gamma$ -secretase. A $\beta$ 1-42 is more prone to  
10 aggregation than A $\beta$ 1-40, is the major component of amyloid plaque (Jarrett et al., 1993, Biochemistry 32:4693-4697; Kuo et al., 1996, J. Biol. Chem. 271:4077-4081), and its production is closely associated with the development of Alzheimer's disease (Sinha & Lieberburg, 1999, Proc. Natl. Acad. Sci. USA 96:11049-11053). The bond cleaved by  $\gamma$ -secretase appears to be situated within the transmembrane domain of  
15 APP. It is unclear as to whether the C-termini of A $\beta$ 1-40 and A $\beta$ 1-42 are generated by a single  $\gamma$ -secretase protease with sloppy specificity or by two distinct proteases. For a review that discusses APP and its processing, see Selkoe, 1998, Trends Cell. Biol. 8:447-453.

Much interest has focused on the possibility of inhibiting the  
20 development of amyloid plaques as a means of preventing or ameliorating the symptoms of Alzheimer's disease. To that end, a promising strategy is to inhibit the activity of  $\beta$ - and  $\gamma$ -secretase, the two enzymes that together are responsible for producing A $\beta$ . This strategy is attractive because, if the formation of amyloid plaques as a result of the deposition of A $\beta$  is a cause of Alzheimer's disease, inhibiting the  
25 activity of one or both of the two secretases would intervene in the disease process at an early stage, before late-stage events such as inflammation or apoptosis occur. Such early stage intervention is expected to be particularly beneficial (see, *e.g.*, Citron, 2000, Molecular Medicine Today 6:392-397).

To that end, various assays have been developed that are directed to the  
30 identification of compounds that may interfere with the production of A $\beta$  or its deposition into amyloid plaques. U.S. Patent No. 5,441,870 is directed to methods of monitoring the processing of APP by detecting the production of amino terminal fragments of APP. U.S. Patent No. 5,605,811 is directed to methods of identifying inhibitors of the production of amino terminal fragments of APP. U.S. Patent No.



5,593,846 is directed to methods of detecting soluble A $\beta$  by the use of binding substances such as antibodies. Esler et al., 1997, *Nature Biotechnology* 15:258-263 described an assay that monitored the deposition of A $\beta$  from solution onto a synthetic analogue of an amyloid plaque. The assay was suitable for identifying compounds  
5 that could inhibit the deposition of A $\beta$ . However, this assay is not suitable for identifying substances, such as inhibitors of  $\beta$ - or  $\gamma$ -secretase, that would prevent the formation of A $\beta$ .

Various groups have cloned and sequenced cDNA encoding a protein that is believed to be  $\beta$ -secretase (Vassar et al., 1999, *Science* 286:735-741; Hussain  
10 et al., 1999, *Mol. Cell. Neurosci.* 14:419-427; Yan et al., 1999, *Nature* 402:533-537; Sinha et al., 1999, *Nature* 402:537-540; Lin et al., 2000, *Proc. Natl. Acad. Sci. USA* 97:1456-1460) but the identity of  $\gamma$ -secretase has been more elusive. A pair of proteins known as presenilin-1 and presenilin-2 are viewed as possible candidates (Selkoe & Wolfe, 2000, *Proc. Natl. Acad. Sci. USA* 97:5690-5692).

15 Presenilin-1 (PS1) and presenilin-2 (PS2) are polytopic membrane proteins that are involved in  $\gamma$ -secretase-mediated processing of APP. The most common cause of familial early-onset Alzheimer's disease is the autosomal dominant inheritance of assorted mutations in the PS1 gene (Sherrington et al., 1995, *Nature* 375:754-760). These PS1 mutations lead to increased production of A $\beta$ 1-42  
20 (Scheuner et al., 1996, *Nature Medicine* 2:864-870; Duff et al., 1996, *Nature* 383:710-713; Borchelt et al., 1996, *Neuron* 17:1005-1013). Similarly, certain mutations in PS2 cause familial early-onset Alzheimer's disease and increased generation of A $\beta$ 42 (Levy-Lahad et al., 1995, *Science* 269:970-973). Cultured isolated neurons from PS1-deficient mice exhibit reduced  $\gamma$ -secretase-mediated  
25 cleavage of APP (De Strooper et al., 1998, *Nature* 391:387-390). It was suggested that PS1 might influence trafficking of APP and/or  $\gamma$ -secretase or it might play a more direct role in proteolytic cleavage of APP. Directed mutagenesis of two conserved transmembrane-situated aspartates in PS1 was shown to inactivate  $\gamma$ -secretase activity in cellular assays, suggesting that PS1 is either a required diasparyl cofactor for  $\gamma$ -  
30 secretase or is itself  $\gamma$ -secretase (Wolfe et al., 1999, *Nature* 398:513-517). Moreover, Li et al., 2000, *Nature* 405:689-694 made photoactivatable derivatives of a highly specific and potent aspartyl protease transition state analog inhibitor and found that the inhibitor selectively labeled presenilin fragments.

Co-immunoprecipitation experiments have shown that PS1 and PS2 interact directly with the immature forms of APP in the endoplasmic reticulum where the disease-associated amyloid A $\beta$ 1-42 peptide is probably generated (Xia et al., 1997 Proc. Natl. Acad. Sci. USA 94:8208-8213; Weidemann et al., 1997, Nat. Med. 3:328-332). Knock-out of PS1 activity greatly diminishes  $\gamma$ -secretase cleavage of APP (De Strooper et al., 1998, Nature 391:387-390). PS1 knock-outs do not exhibit total lack of  $\gamma$ -secretase activity but knock-out of both PS1 and PS2 activity does result in a total loss of  $\gamma$ -secretase activity (Herreman et al., 2000, Nat. Cell. Biol. 2:461-462; Zhang et al., 2000, Nat. Cell Biol. 2:463-465), suggesting that PS2 has a similar function to PS1 in the processing of APP.

Karlström et al., (Journal of Biological Chemistry papers in press, published on December 13, 2001 as Manuscript C100649200) describes an assay designed specifically to identify inhibitors of  $\gamma$ -secretase cleavage of APP. The authors inserted the GAL4 DNA binding domain fused to the VP16 transactivation domain into C99, a portion of APP containing the 99 carboxy-terminal amino acids. This fragment of APP contains the  $\gamma$ -secretase cleavage site but lacks the  $\beta$ -secretase cleavage site. Transfection of a UAS reporter plasmid by GAL4-VP16 confirmed cleavage of the Gal4-VP16/C99 substrate by  $\gamma$ -secretase only. Thus, the assay is capable of detecting  $\gamma$ -secretase inhibitors but not inhibitors of  $\beta$ -secretase or other modulators of APP processing requiring the N-terminal domain of APP.

Cao & Südhoff, 2001, Science 293:115-120 described work in which the GAL4 and LexA DNA binding domains were inserted into APP to demonstrate the potential of the cleaved C-terminus of APP for transcriptional co-activation. In this article, a transcriptional factor was not fused to APP and no attempt was made to develop an assay for the identification of APP processing inhibitors.

Sisodia, 1992, Proc. Natl. Acad. Sci. USA 89:6975-6979 described various changes in the amino acid sequence of APP in the region of the  $\alpha$ -secretase cleavage site and the effect of those changes on cleavage by  $\alpha$ -secretase. A change of K to V at position 612 of the 695 amino acid version of APP led to reduced cleavage by  $\alpha$ -secretase.

U.S. Patent No. 6,333,167 B1 discloses an assay involving DNA constructs encoding portions of membrane proteins containing sites that are susceptible to cleavage by proteases that are fused to transcriptional repressors. Such constructs are introduced into cells that contain a reporter gene under the control of a

promoter that is sensitive to the repressor. In the absence of an inhibitor of the protease, the fusion protein is cleaved by the protease, releasing a membrane protein/repressor fusion protein that translocates to the nucleus and represses transcription from the reporter gene. In the presence of an inhibitor of the protease,  
5 the membrane protein/repressor fusion protein is not released and thus cannot repress transcription from the reporter. An increase in reporter expression can therefore be used as a readout for the presence of an inhibitor.

#### SUMMARY OF THE INVENTION

10 The present invention is directed to methods of identifying inhibitors of the processing of amyloid precursor protein (APP) that are capable of identifying inhibitors of a number of steps of such processing. Unlike prior methods, the methods of the present invention can be used to screen for inhibitors of  $\beta$ -secretase cleavage,  $\gamma$ -secretase cleavage, APP extracellular signaling, or APP cytoplasmic signaling in a  
15 single assay.

The methods employ a recombinant eukaryotic cell that is capable of processing APP. The cell has been engineered to express a fusion protein that contains amino acid sequences encompassing both the  $\beta$ -secretase cleavage site of APP and the  $\gamma$ -secretase cleavage site. The fusion protein also contains a transcription  
20 factor fused in frame to the APP sequences.

When the recombinant cell is further engineered to contain a reporter gene, in which transcription of the reporter gene is driven by a regulatory DNA sequence that is inactive in the absence of the transcription factor but active in the transcription factor's presence, a system useful for screening for APP processing  
25 inhibitors is provided. Since the recombinant cell has been selected so as to be capable of processing APP, the fusion protein will be processed, releasing the transcription factor and activating transcription of the reporter gene. The reporter gene has been preselected so that activation of the reporter gene leads to a detectable phenotype.

30 The system is utilized by exposing the recombinant cell to substances that are to be tested for the ability to inhibit APP processing. Those substances that are actually inhibitors of APP processing will cause diminished processing of the fusion protein, leading to smaller amounts of the transcription factor being released.

This leads to less transcription of the reporter gene. This results in a decrease in the phenotypic effect of the reporter gene that can be observed.

#### BRIEF DESCRIPTION OF THE DRAWINGS

5                   Figure 1A-G shows a schematic diagram of several APP/transcription factor fusion constructs.

Figure 2A-B shows the DNA sequence (SEQ ID NO:1) of the fusion protein APP(1-651)SW, K612V-TATexonI(M1L) APP (664-695).

10                   Figure 3 shows the amino acid sequence (SEQ ID NO:2) of the fusion protein APP(1-651)SW, K612V-TATexonI(M1L) APP (664-695) with the various different regions of the fusion protein demarcated. 1 = amino acids 1-651 of APP; 2 = region of  $\beta$ -secretase cleavage; 3 = K612V mutation; 4 = region of  $\gamma$ -secretase cleavage; 5 = linker; 6 = TAT exon I; 7 = linker; 8 = amino acids 664-695 of APP.

15                   Figure 4A-B shows the DNA sequence (SEQ ID NO:3) of the fusion protein APP(1-651)wt, K612V-TATexonI(M1L) APP (664-695).

20                   Figure 5 shows the amino acid sequence (SEQ ID NO:4) of the fusion protein APP(1-651)wt, K612V-TATexonI(M1L) APP (664-695) with the various different regions of the fusion protein demarcated. 1 = amino acids 1-651 of APP; 2 = region of  $\beta$ -secretase cleavage; 3 = K612V mutation; 4 = region of  $\gamma$ -secretase cleavage; 5 = linker; 6 = TAT exon I; 7 = linker; 8 = amino acids 664-695 of APP.

Figure 6A-C shows the DNA sequence (SEQ ID NO:5) of the fusion protein APP(1-651)SW, K612V, GAL4-VP16(delMet) APP (664-695).

25                   Figure 7 shows the amino acid sequence (SEQ ID NO:6) of the fusion protein APP(1-651)SW, K612V, GAL4-VP16(delMet) APP (664-695) with the various different regions of the fusion protein demarcated. 1 = amino acids 1-651 of APP; 2 = region of  $\beta$ -secretase cleavage; 3 = K612V mutation; 4 = region of  $\gamma$ -secretase cleavage; 5 = linker; 6 = GAL4-VP16; 7 = linker; 8 = amino acids 664-695 of APP.

30                   Figure 8A-C shows the DNA sequence (SEQ ID NO:7) of the fusion protein APP(1-651)wt, K612V, GAL4-VP16(del Met) APP (664-695).

Figure 9 shows the amino acid sequence (SEQ ID NO:8) of the fusion protein APP(1-651)wt, K612V, GAL4-VP16(del Met) APP (664-695) with the various different regions of the fusion protein demarcated. 1 = amino acids 1-651 of APP; 2 = region of  $\beta$ -secretase cleavage; 3 = K612V mutation; 4 = region of  $\gamma$ -

secretase cleavage; 5 = linker; 6 = GAL4-VP16; 7 = linker; 8 = amino acids 664-695 of APP.

Figure 10A-B shows the DNA sequence (SEQ ID NO:9) of the fusion protein APP(1-651)SW, TATexonI(M1L) APP (664-695).

5           Figure 11 shows the amino acid sequence (SEQ ID NO:10) of the fusion protein APP(1-651)SW, TATexonI(M1L) APP (664-695) with the various different regions of the fusion protein demarcated. 1 = amino acids 1-651 of APP; 2 = region of  $\beta$ -secretase cleavage; 3 = wild-type K at position 612; 4 = region of  $\gamma$ -secretase cleavage; 5 = linker; 6 = TAT exon I; 7 = linker; 8 = amino acids 664-695 of  
10 APP.

Figure 12A-B shows the DNA sequence (SEQ ID NO:11) of the fusion protein APP(1-651)wt, TATexonI(M1L) APP (664-695).

Figure 13 shows the amino acid sequence (SEQ ID NO:12) of the fusion protein APP(1-651)wt, TATexonI(M1L) APP (664-695) with the various  
15 different regions of the fusion protein demarcated. 1 = amino acids 1-651 of APP; 2 = region of  $\beta$ -secretase cleavage; 3 = wild-type K at position 612; 4 = region of  $\gamma$ -secretase cleavage; 5 = linker; 6 = TAT exon I; 7 = linker; 8 = amino acids 664-695 of APP.

Figure 14A-C shows the DNA sequence (SEQ ID NO:13) of the fusion  
20 protein APP(1-651)SW, GAL4-VP16(delMet) APP (664-695).

Figure 15 shows the amino acid sequence (SEQ ID NO:14) of the fusion protein APP(1-651)SW, GAL4-VP16(delMet) APP (664-695) with the various different regions of the fusion protein demarcated. 1 = amino acids 1-651 of APP; 2 = region of  $\beta$ -secretase cleavage; 3 = wild-type K at position 612; 4 = region of  $\gamma$ -secretase cleavage; 5 = linker; 6 = GAL4-VP16; 7 = linker; 8 = amino acids 664-695  
25 of APP.

Figure 16A-C shows the DNA sequence (SEQ ID NO:15) of the fusion protein APP(1-651)wt, GAL4-VP16(delMet) APP (664-695).

Figure 17 shows the amino acid sequence (SEQ ID NO:16) of the  
30 fusion protein APP(1-651)wt, GAL4-VP16(delMet) APP (664-695) with the various different regions of the fusion protein demarcated. 1 = amino acids 1-651 of APP; 2 = region of  $\beta$ -secretase cleavage; 3 = wild-type K at position 612; 4 = region of  $\gamma$ -secretase cleavage; 5 = linker; 6 = GAL4-VP16; 7 = linker; 8 = amino acids 664-695 of APP.

Figure 18A-B shows the cDNA sequence (SEQ ID NO:17) and Figure 18C shows the amino acid sequence (SEQ ID NO:18) of the 695 amino acid splice variant of wild-type Alzheimer's precursor protein (APP). See GenBank accession no. Y00264 and Kang et al., 1987, Nature 325:733-736.

5                    Figure 19 shows data from an embodiment in which the assay of the present invention was used to identify both a  $\beta$ -secretase inhibitor and a  $\gamma$ -secretase inhibitor. See Example 3 for details.

Figure 20 shows a schematic diagram of pCR2.1 Gal4-VP16.

10                   Figure 21A shows a schematic diagram of pRBR121. Figure 21B shows a schematic diagram of pcDNA3.1 zeo (+) APP(1-651)SW, K612V-TATexonI(M1L) APP (664-695).

15                   Figure 22A shows a schematic diagram of pRBR186. Figure 22B shows a schematic diagram of the viral plasmid pNL4-3. Figure 22C shows a schematic diagram of pcDNA3.1 zeo (+) APP(1-651)SW, K612V-TATexonI(M1L) APP (664-695) with additional details as compared to Figure 21B, which shows the same plasmid.

Figure 23 shows a schematic diagram of pRSV Kan/Neo res.

Figure 24 shows a schematic diagram of pUCd5TAT.

20                   Figure 25A shows a schematic diagram of pMM321. Figure 25B-D shows the nucleotide sequence of pMM321. The upper strand is SEQ ID NO:19. The lower strand (SEQ ID NO:20) is the reverse complement of SEQ ID NO:19.

25                   Figure 26A shows a schematic diagram of the expression vector pcDNA3.1 zeo (+)APP(1-651)SW, K612V-(M1L)TATexonI. This expression vector directs the expression of a fusion protein containing the first 651 amino acids of APP with the Swedish version of the  $\beta$ -secretase cleavage site and the K612V mutation fused to the first exon of HIV1 TAT. The methionine at position 1 of TAT has been changed to leucine. Figure 26B-G shows the nucleotide sequence of pcDNA3.1 zeo (+)APP(1-651)SW, K612V-(M1L)TATexonI. The upper strand is SEQ ID NO:21. The lower strand (SEQ ID NO:22) is the reverse complement of SEQ ID NO:21.

30                   Figure 27A-B shows a schematic diagram depicting general features of the present invention. Figure 27A: The vertical bar represents a fusion protein with APP sequences represented as unfilled or lightly shaded portions of the bar. The lightly shaded portion represents A $\beta$ . "BACE" indicates the  $\beta$ -secretase cleavage site. The dark shaded portion represents the transcription factor fused between APP

sequences. The horizontal bar represents a membrane in which the uncleaved fusion protein is embedded, e.g., the endoplasmic reticulum. Figure 27B: The transcription factor (plus small amounts of APP), having been released from the fusion protein and thus the membrane by APP processing, is shown in the nucleus binding to and  
 5 activating the regulatory DNA sequence ("Transcription Factor Response Element") that controls the expression of the reporter gene.

Figure 28A-B shows the DNA sequence (SEQ ID NO:23) of the fusion protein APP(1-651)NFEV, K612V-TATexonI(M1L) APP (664-695).

Figure 29 shows the amino acid sequence of a fusion protein (APP(1-  
 10 651)NFEV, K612V-TATexonI(M1L) APP (664-695)) (SEQ ID NO:24) containing the sequence NFEV at the  $\beta$ -secretase cleavage site (underlined at 2). The other portions of the fusion protein are indicated as follows: 1 = amino acids 1-651 of APP; 2 = region of  $\beta$ -secretase cleavage; 3 = K612V mutation; 4 = region of  $\gamma$ -secretase cleavage; 5 = linker; 6 = TAT exon I; 7 = linker; 8 = amino acids 664-695 of APP.

Figure 30A-C shows the DNA sequence (SEQ ID NO:25) of the fusion  
 15 protein APP(1-651)NFEV, K612V, GAL4-VP16(delMet) APP (664-695).

Figure 31 shows the amino acid sequence of a fusion protein (APP(1-  
 20 651)NFEV, K612V, GAL4-VP16(delMet) APP (664-695)) (SEQ ID NO:26) containing the sequence NFEV at the  $\beta$ -secretase cleavage site (underlined at 2). The other portions of the fusion protein are indicated as follows: 1 = amino acids 1-651 of APP; 2 = region of  $\beta$ -secretase cleavage; 3 = K612V mutation; 4 = region of  $\gamma$ -secretase cleavage; 5 = linker; 6 = GAL4-VP16; 7 = linker; 8 = amino acids 664-695 of APP.

Figure 32A shows a schematic diagram of pcDNA3.1 zeo (+), a  
 25 eukaryotic expression vector that is suitable for use in the present invention. Figure 32B-F shows the nucleotide sequence of pcDNA3.1 zeo (+). The upper strand is SEQ ID NO:27. The lower strand (SEQ ID NO:28) is the reverse complement of SEQ ID NO:27.

Figure 33 shows data from an embodiment of the present invention  
 30 utilizing a  $\beta$ -galactosidase reporter gene in which the assay of the present invention was used to identify both a  $\beta$ -secretase inhibitor and a  $\gamma$ -secretase inhibitor. See Example 8 for details.

Figure 34 shows data from an embodiment of the present invention in which a fusion protein having a wild-type  $\beta$ -secretase cleavage site and a fusion

protein having a Swedish  $\beta$ -secretase cleavage site are compared. See Example 9 for details.

Figure 35A-B shows the DNA sequence (SEQ ID NO:29) of the fusion protein APP(1-651)NFEV, TATexonI(M1L) APP (664-695).

5           Figure 36 shows the amino acid sequence of a fusion protein (APP(1-651)NFEV, TATexonI(M1L) APP (664-695)) (SEQ ID NO:30) containing the sequence NFEV at the  $\beta$ -secretase cleavage site (underlined at 2) and a wild-type K at position 612 (underlined at 3). The other portions of the fusion protein are indicated as follows: 1 = amino acids 1-651 of APP; 2 = region of  $\beta$ -secretase cleavage; 3 =  
10       wild-type K; 4 = region of  $\gamma$ -secretase cleavage; 5 = linker; 6 = TAT exon I; 7 = linker; 8 = amino acids 664-695 of APP.

Figure 37A-C shows the DNA sequence (SEQ ID NO:31) of the fusion protein APP(1-651)NFEV, GAL4-VP16(delMet) APP (664-695).

15           Figure 38 shows the amino acid sequence of a fusion protein (APP(1-651)NFEV, GAL4-VP16(delMet) APP (664-695)) (SEQ ID NO:32) containing the sequence NFEV at the  $\beta$ -secretase cleavage site (underlined at 2) and a wild-type K at position 612 (underlined at 3). The other portions of the fusion protein are indicated as follows: 1 = amino acids 1-651 of APP; 2 = region of  $\beta$ -secretase cleavage; 3 =  
wild-type K; 4 = region of  $\gamma$ -secretase cleavage; 5 = linker; 6 = GAL4-VP16; 7 =  
20       linker; 8 = amino acids 664-695 of APP.

## DETAILED DESCRIPTION OF THE INVENTION

For the purposes of this invention:

25       A "fusion protein" is a protein that contains at least two polypeptide regions and, optionally, a linking peptide to operatively link the two polypeptides into one continuous polypeptide. The at least two polypeptide regions in a fusion protein are derived from different sources, and therefore a fusion protein comprises two polypeptide regions not normally joined together in nature.

30       A "linking sequence (or linker peptide)" contains one or more amino acid residues joined in peptide bonds. A linking sequence serves to join two polypeptide regions of differing origins in a fusion protein via a peptide bond between the linking sequence and each of the polypeptide regions.

Typically, a fusion protein is synthesized as a continuous polypeptide in a recombinant host cell which contains an expression vector comprising a



nucleotide sequence encoding the fusion protein where the different regions of the fusion protein are fused in frame on either side of a linker peptide's coding sequence. The chimeric coding sequence (encoding the fusion protein) is operatively linked to expression control sequences (generally provided by the expression vector) that are functional in the recombinant host cell.

"Reporter gene," as used in the present invention, does not mean a DNA sequence present on the chromosome of a cell, generally possessing introns, as is often meant by the word "gene" in the art. Rather "reporter gene" means any DNA sequence encoding a protein or polypeptide that can give rise to a signal that can be detected or measured. "Reporter gene" does not mean a portion of the amino acid sequence of APP. "Reporter gene" will usually mean a DNA sequence, generally a cDNA sequence (although in some cases a reporter gene may have introns) that encodes a protein or polypeptide that is commonly used in the art to provide a measurable phenotype that can be distinguished over background signals.

A "nuclear localization signal (NLS)" is a region of a polypeptide which targets the polypeptide to the nucleus of the cell. One such NLS is that from the SV40 large T antigen. See, e.g., U.S. Patent No. 5,589,392; Kalderon et al., 1984, Cell 39:499-509. The minimum region of the SV40 large T antigen with NLS activity is Pro-Lys-Lys-Lys-Arg-Lys-Val (SEQ ID NO:22). See also U.S. Patent No. 5,776,689.

"Substances" that are screened in the present invention can be any substances that are generally screened in the pharmaceutical industry during the drug development process. For example, substances may be low molecular weight organic compounds (*e.g.*, having a molecular weight of less than about 2,000 daltons and preferably less than about 1,000 daltons), RNA, DNA, antibodies, peptides, or proteins. Substances are often tested in the methods of the present invention as large collections of substances, *e.g.* libraries of low molecular weight organic compounds, peptides, or natural products.

The conditions under which substances are employed in the methods described herein are conditions that are typically used in the art for the study of protein-ligand interactions or enzyme inhibition studies: *e.g.*, salt conditions such as those represented by such commonly used buffers as PBS or in tissue culture media; a temperature of about 4°C to about 55°C; incubation times of from several seconds to several hours or even up to 24 or 48 hours. Screening for the identification of

enzyme-specific inhibitors is a well-known procedure in the pharmaceutical arts and the numerous conditions under which such screening has been done are available in the literature to guide the practitioner of the present invention.

A "conservative amino acid substitution" refers to the replacement of one amino acid residue by another, chemically similar, amino acid residue. Examples of such conservative substitutions are: substitution of one hydrophobic residue (isoleucine, leucine, valine, or methionine) for another; substitution of one polar residue for another polar residue of the same charge (*e.g.*, arginine for lysine; glutamic acid for aspartic acid); substitution of one aromatic amino acid (tryptophan, tyrosine, or phenylalanine) for another.

"Transfection" refers to any of the methods known in the art for introducing DNA into a cell, *e.g.*, calcium phosphate or calcium chloride mediated transfection, electroporation, infection with a retroviral vector.

The present invention relates to the discovery of an assay system that permits the simultaneous screening for inhibitors of several types of amyloid precursor protein (APP) processing or signaling (*e.g.*,  $\beta$ -secretase cleavage,  $\gamma$ -secretase cleavage, APP extracellular signaling, APP cytoplasmic signaling). In a preferred embodiment, this screening is accomplished without the concomitant identification of inhibitors of  $\alpha$ -secretase. The assay system is carried out in a single type of cell, using a single type of assay readout. Inhibitors discovered by means of the present invention are expected to be useful in the treatment of Alzheimer's disease since these inhibitors are likely to be capable of interfering with the production of A $\beta$ .

Previous assays for identifying inhibitors of APP processing have focussed specifically on inhibition of either  $\beta$ -secretase or  $\gamma$ -secretase activity, or on inhibition of some other single aspect of A $\beta$  production. In contrast, the assays described herein are directed to inhibition of APP processing in general. Substances identified through these assays may target  $\beta$ -secretase,  $\gamma$ -secretase, modulators of  $\beta$ -secretase or  $\gamma$ -secretase activity, or even an as-yet-undiscovered ligand interaction with APP. In certain embodiments, these assays will also be free of the potentially misleading or obscuring effects of  $\alpha$ -secretase activity. In addition, unlike other assays currently in use, these assays are homogeneous assays; *i.e.*, they require no cumbersome or time-consuming steps such as column chromatography separations, immunoprecipitations, washing steps, etc. Therefore, the assays are very well adapted to a high throughput screening format.

In the present invention, novel recombinant DNA molecules are constructed in which nucleotide sequences encoding at least a portion of the luminal (i.e., N-terminal to the transmembrane region) and transmembrane regions of APP are fused to nucleotide sequences encoding a transcription factor. In a preferred  
5 embodiment, the APP contains an  $\alpha$ -secretase cleavage site that has been altered to reduce or eliminate  $\alpha$ -secretase cleavage. This allows the assays of the present invention to avoid identifying inhibitors of  $\alpha$ -secretase and permits the more efficient detection of  $\beta$ -secretase inhibitors since  $\alpha$ -secretase and  $\beta$ -secretase compete for APP cleavage. The recombinant DNA molecules may be transfected, along with a reporter  
10 gene, into a cell line that processes APP into A $\beta$ , and stable clones may be generated. Alternatively, the recombinant DNA molecules and reporter plasmid may be utilized in transient transfections.

Upon expression in cells, the APP/transcription factor fusion protein localizes to a non-nuclear membrane of the cell (e.g., the endoplasmic reticulum) due  
15 to the presence of the APP sequences in the fusion protein. In a manner similar to cleavage of APP, the fusion protein will then be cleaved, first by  $\beta$ -secretase and then by  $\gamma$ -secretase.  $\gamma$ -secretase cleavage releases the transcription factor from the membrane in which the APP/transcription factor fusion protein had been embedded, after which the transcription factor translocates to the nucleus and stimulates  
20 transcription of the reporter gene. Assuming no  $\alpha$ -secretase cleavage, cleavage by both  $\beta$ -secretase and  $\gamma$ -secretase is required for release of the transcription factor and transactivation of the reporter gene in this assay since  $\gamma$ -secretase cleavage of APP is dependent on a short luminal domain, such as that generated by  $\beta$ - or  $\alpha$ -secretase cleavage. Detection of a signal from the reporter gene product will thus serve as  
25 evidence of APP processing. In particular, since activation of the reporter gene requires both  $\beta$ -secretase and  $\gamma$ -secretase cleavage, the assay is capable of identifying inhibitors of both or either of these proteases.

Figure 27 is a schematic diagram depicting general features of the assay. The vertical bar in Figure 27A represents the fusion protein; the horizontal bar  
30 represents the non-nuclear membrane in which the fusion protein is embedded before processing. Figure 27B shows how the transcription factor portion of the fusion protein (with small amounts of the APP portion flanking it) has moved to the nucleus following release from the fusion protein by APP processing. In the nucleus, the

transcription factor is shown binding to a regulatory DNA sequence ("Transcription Factor Response Element") and activating transcription of the reporter gene.

The recombinant DNA molecules encoding the APP/transcription factor fusion protein and the reporter gene can be used to develop novel homogenous cell-based assays for the identification and assessment of inhibitors of APP processing which will be readily amenable to high throughput technology.

In one embodiment, the recombinant DNA molecules used in this invention comprise sequences encoding the amino terminal 651 amino acids of the 695 amino acid version of APP (Kang et al., 1987, Nature 325:733-736), including all the sequences necessary for the production of A $\beta$ , as well as the C-terminal 32 amino acids of APP. The transcription factor is placed between the N-terminal and C-terminal portions of APP. The APP sequence may include a modification to increase the amount of  $\beta$ -secretase cleavage of the fusion protein. This modification involves mutating the K at position 612 of the  $\alpha$ -secretase cleavage site to a V (K612V). Since  $\alpha$ -secretase and  $\beta$ -secretase compete for APP cleavage, reducing or eliminating APP cleavage by  $\alpha$ -secretase results in increased  $\beta$ -secretase cleavage, and allows the assay to detect  $\beta$ -secretase inhibitors more readily. In addition, the  $\beta$ -secretase cleavage site within APP (KM↓DA) (SEQ ID NO:34) may be modified, e.g., to that of a naturally occurring mutation (termed the "Swedish" mutation or NL↓DA) (SEQ ID NO:38) which has been shown to enhance  $\beta$ -secretase cleavage six-fold in cultured cells. Another possible modification is to replace the (KM↓DA) (SEQ ID NO:34) wild-type  $\beta$ -secretase cleavage site with the sequence (NF↓EV) (SEQ ID NO:40). The presence of NFEV in an amino acid sequence has been shown to enhance  $\beta$ -secretase cleavage by an even larger amount than the Swedish sequence. See U.S. Provisional Patent Application Serial No. 60/292,591 and U.S. Provisional Patent Application Serial No. 60/316,115, the disclosures of which are incorporated herein, in their entirety.

In a preferred embodiment, HIV-1 TAT exon I has been fused between sequences encoding the first 651 amino acids of APP<sub>695</sub> and the last 32 amino acids of APP<sub>695</sub> (APP-TAT-APPct32). Co-transfection of an expression vector comprising this construct with a reporter gene plasmid containing an HIV-1 LTR promoter that controls the transcription of a reporter gene leads to enhanced expression of the reporter gene. Other transcription factors that could be fused to APP<sub>1-651</sub> include Gal4-VP16, the entire Gal4 protein, BIV TAT, HIV-2 TAT, SIV TAT, LexA-VP16, EBV Zta, Papillomavirus E2, or tissue or species specific homodimeric bHLH

transcription factors capable of activating transcription through specific DNA response elements, such as E12, E47, or Twist. The use of GAL4, BIV, HIV-2, or SIV TAT may be useful if it is desired to reduce the potency of the transactivator, thus reducing any background transactivation caused by non-specific cleavage of the fusion protein. To further reduce the potential for transactivation by TAT in the absence of  $\beta$ -secretase and  $\gamma$ -secretase cleavage, the TAT portion of the fusion protein may be altered to remove the N-terminal methionine and thus eliminate the possibility of aberrant translation of TAT through any potential internal ribosomal entry sites.

In some circumstances, high level expression of TAT has been found to be toxic to cells. Thus, when TAT is the transcription factor fused to APP in the methods of the present invention, it may be advantageous to utilize transient transfection with low amounts of the expression vector encoding the APP/TAT fusion protein. A set of preliminary experiments in which various amounts of the vector are transfected, in order to titrate acceptable levels of TAT, is recommended.

The reporter gene used will depend in large part upon the transcription factor fused to APP. The promoter used to drive the reporter gene will be LTR for TAT-based APP fusion proteins, or UAS (6x) for GAL4-VP16-based APP fusion proteins. In a particular embodiment, an LTR driving EGFP (enhanced green fluorescent protein, a brighter variant of GFP made by Aurora Biosciences, San Diego, CA) has been used to observe processing of an APP/TAT fusion protein. Under certain conditions, it may be desirable to use a less stable reporter, such as dsEGFP (a destabilized variant of EGFP made by Aurora Biosciences, San Diego, CA and marketed by Clontech, Palo Alto, CA) or a more potent reporter, such as  $\beta$ -lactamase. Alternatively, a stable HeLa cell line expressing LTR- $\beta$ -galactosidase can be used. If the exquisite sensitivity of  $\beta$ -lactamase makes it less than optimal for a particular purpose, the LTR- $\beta$ -galactosidase cell line may be exploited for this assay. Finally, under some circumstances Gal4-VP16 may prove to be optimal relative to TAT to reduce any inherent background problems associated with using the weakly but constitutively active LTR in the reporter plasmid, in which case the reporter plasmid could be UAS(6x)- $\beta$ -lactamase (Aurora Biosciences, San Diego, CA).

A variety of cells are suitable for use in the methods of the present invention. Particularly preferred are eukaryotic, especially mammalian, cell lines. In particular embodiments, the cells are selected from the group consisting of: L cells L-M(TK-) (ATCC CCL 1.3), L cells L-M (ATCC CCL 1.2), HEK293 (ATCC CRL

1573), HEK293T, Raji (ATCC CCL 86), CV-1 (ATCC CCL 70), COS-1 (ATCC CRL 1650), COS-7 (ATCC CRL 1651), CHO-K1 (ATCC CCL 61), 3T3 (ATCC CCL 92), NIH/3T3 (ATCC CRL 1658), HeLa (ATCC CCL 2), C1271 (ATCC CRL 1616), BS-C-1 (ATCC CCL 26), T24 (ATCC HTB-4), PC12 cells, Jurkat cells, H4 cells (ATCC HTB-148), and MRC-5 (ATCC CCL 171).

To make the assay more amenable for ultra-high throughput screening, a non-adherent cell line, such as Jurkat, can be used.

Generally, the assays of the present invention employ cells that naturally express  $\beta$ -secretase and  $\gamma$ -secretase. However, it is possible to practice the invention in cells that lack the expression of one, or both, of these enzymes. In such cases,  $\beta$ -secretase and  $\gamma$ -secretase activity can be provided by the recombinant expression of these enzymes in the cells.

In one embodiment, the present invention provides a recombinant cell, preferably a eukaryotic cell, even more preferably a mammalian cell, and most preferably a human cell, where the cell expresses a fusion protein of APP and a transcription factor and the cell contains a reporter gene that can be activated by the transcription factor. The fusion protein comprises a portion of APP where that portion includes the regions of the  $\beta$ -secretase and  $\gamma$ -secretase cleavage sites fused to a transcription factor. The region of APP including the  $\beta$ -secretase and  $\gamma$ -secretase cleavage sites can be, e.g., a portion of APP that includes amino acids 589-651 of the 695 amino acid version of APP. This region is shown below.

↓
 ↓ ↓  
 EEISEVKM DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVV IA  
 25 TVIVITLVMKKK (SEQ ID NO:33)

The  $\beta$ -secretase cleavage site is shown at position 596-597 (KM DA) (SEQ ID NO:34).

Two predominant cleavage sites of  $\gamma$ -secretase are shown at positions 636-637 and 638-639

↓   ↓  
(GVV 1A TV) (SEO ID NO>35).

The fusion protein will be anchored in the membrane by the APP  
35 sequences shown above. The N-terminal portion of APP must include at least the  $\beta$ -

secretase cleavage site, and possibly several amino-acids N-terminal to the  $\beta$ -secretase cleavage site to make the assay sensitive to both  $\beta$ -secretase and  $\gamma$ -secretase inhibitors. In many cases, the APP sequences will include sequences further N-terminal than those shown above, including the signal sequence at the N-terminus of APP. In cases, where the APP signal sequence is not used, another signal sequence may be incorporated in the fusion protein. Such other signal sequences are known in the art.

In a related embodiment, the present invention provides a recombinant cell, preferably a eukaryotic cell, even more preferably a mammalian cell, and most preferably a human cell, where the above-described APP portion of the fusion protein contains a K612V mutation. The APP portion of this embodiment is shown below.

↓ ↓ ↓  
EEISEVKM DAEFRHDSGYEVHHQVLVFFAEDVGSNKGAIIGLMVGGVV IA  
TVIVITLVMLKKK (SEQ ID NO:36)

The  $\beta$ -secretase cleavage site is shown at position 596-597 (KM DA) (SEQ ID NO:34).

Two predominant cleavage sites of  $\gamma$ -secretase are shown at positions 636-637 and 638-639

↓ ↓  
(GVV LA TV) (SEQ ID NO:35).

The underlined V at position 612 shows the change in sequence in the present invention from the wild-type K to the mutant V, which change provides for reduced cleavage by  $\alpha$ -secretase.

In a related embodiment, the present invention provides a recombinant cell, preferably a eukaryotic cell, even more preferably a mammalian cell, and most preferably a human cell, where the above-described APP portion of the fusion protein contains the Swedish version of the  $\beta$ -secretase cleavage site as well as a K612V mutation. The APP portion of this embodiment is shown below.

↓ ↓ ↓  
EEISEVNL DAEFRHDSGYEVHHQVLVFFAEDVGSNKGAIIGLMVGGVV IA  
TVIVITLVMLKKK (SEQ ID NO:37)

The  $\beta$ -secretase cleavage site is shown at position 596-597 (NL DA) (SEQ ID NO:38).

Two predominant cleavage sites of  $\gamma$ -secretase are shown at positions 636-637 and 638-639

↓ ↓

(GVV 1A TV) (SEQ ID NO:35).

5 The underlined V at position 612 shows the change in sequence in the present invention from the wild-type K to the mutant V, which change provides for reduced cleavage by  $\alpha$ -secretase.

In a related embodiment, the present invention provides a recombinant cell, preferably a eukaryotic cell, even more preferably a mammalian cell, and most preferably a human cell, where the above-described APP portion of the fusion protein contains the NFEV version of the  $\beta$ -secretase cleavage site as well as a K612V mutation. The APP portion of this embodiment is shown below.

↓

15 EEISEVNF EVEFRHDSGYEVHHQYL<sup>VFF</sup>FAEDVGSNKGAIIGLMVGGVV IA  
TVIVITLVMLKKK (SEQ ID NO:39)

↓ ↓

4

The  $\beta$ -secretase cleavage site is shown at position 596-597 (NF EV) (SEQ ID NO:40).

20 Two predominant cleavage sites of  $\gamma$ -secretase are shown at positions 636-637 and 638-639

↓ ↓

(GVV 1A TV) (SEQ ID NO:35).

25 The underlined V at position 612 shows the change in sequence in the present invention from the wild-type K to the mutant V, which change provides for reduced cleavage by  $\alpha$ -secretase.

The presence of both  $\beta$ -secretase and  $\gamma$ -secretase cleavage sites in the fusion proteins permits the assays of the present invention to detect inhibitors of both  $\beta$ -secretase and  $\gamma$ -secretase.

30 The recombinant host cells of the present invention can be further engineered to comprise a reporter gene construct. The reporter gene construct contains a reporter gene in operable linkage with a regulatory DNA sequence that confers on the reporter gene the property of being regulated by the transcription factor of the fusion protein. This regulation is such that expression of the reporter gene is  
35 low or absent without binding of the transcription factor to the regulatory DNA



sequence but, when the transcription factor is released from the fusion protein by APP processing, the transcription factor can move into the nucleus of the cell and bind to the regulatory DNA sequence, thereby activating transcription from the reporter gene.

Reporter genes desirably give rise to gene products which can be  
5 detected or quantitated, either in terms of amount of protein synthesized, enzymatic activity, fluorescence, luminescence, or some other phenotype. Suitable reporter gene products include firefly luciferase (de Wet et al., 1987, Mol. Cell. Biol. 7:725-737) or bacterial luciferase (Englebrecht et al., 1985, Science 227:1345-1347; Baldwin et al., 1984, Biochem. 23:3663-3667),  $\beta$ -lactamase,  $\beta$ -glucuronidase,  $\beta$ -galactosidase, green  
10 fluorescent proteins, enhanced green fluorescent protein, destabilized enhanced green fluorescent protein, red fluorescent protein, yellow fluorescent protein, cyan fluorescent protein, destabilized yellow fluorescent protein, destabilized cyan fluorescent protein, aequorin, chloramphenicol acetyl transferase (Alton & Vapnek, 1979, Nature 282:864-869), rat liver alkaline phosphatase (Toh et al., 1989, Eur. J.  
15 Biochem. 182:231-237), human placental secreted alkaline phosphatase (Cullen & Mallim, 1992, Meth. Enzymol. 216:362-368), and horseradish peroxidase, among others.

A preferred reporter gene is green fluorescent protein (GFP) or a modified GFP. Wild-type GFP has long been used in the art. Starting from green  
20 fluorescent protein, many modified versions have been derived with altered or enhanced spectral properties as compared with wild-type GFP. See, *e.g.*, U.S. Patent No. 5,625,048; International Patent Publication WO 97/28261; International Patent Publication WO 96/23810. Useful are the modified GFPs W1B and TOPAZ, available commercially from Aurora Biosciences Corp., San Diego, CA. W1B  
25 contains the following changes from the wild-type GFP sequence: F64L, S65T, Y66W, N146I, M153T, and V163A (see Table 1, page 519, of Tsien, 1998, Ann. Rev. Biochem. 67:509-544). TOPAZ contains the following changes from the wild-type GFP sequence: S65G, V68L, S72A, and T203Y (see Table 1, page 519, of Tsien, 1998, Ann. Rev. Biochem. 67:509-544). Wild-type nucleotide and amino acid  
30 sequences of GFP are shown in Figure 1 and SEQ ID NO: 1 of International Patent Publication WO 97/28261; in Figure 1 of Tsien, 1998, Ann. Rev. Biochem. 67:509-544; and in Prasher et al., 1992, Gene 111:229-233.

When expressing GFPs in mammalian cells, it may be advantageous to construct versions of the GFPs having altered codons that conform to those codons

preferred by mammalian cells (Zolotukhin et al., J. Virol. 1996, 70:4646-46754; Yang et al., 1996, Nucl. Acids Res. 24:4592-4593). Another way of improving GFP expression in mammalian cells is to provide an optimal ribosome binding site by the use of an additional codon immediately after the starting methionine (Cramer et al., 5 1996, Nature Biotechnology 14:315-319).

Transcription factors that are useful in the present invention are preferably those transcription factors that are not naturally expressed in the recombinant host cells. This is so the regulatory DNA sequence is not activated absent APP processing and release of the transcription factor from the fusion protein. 10 Preferably, the transcription factor contains, or is engineered to contain, a nuclear localization signal. This is so that, after release, the transcription factor will move into the nucleus of the genetically modified host cells where it can bind to, and activate, the regulatory DNA sequence, leading to expression of the reporter gene. Transcription factors, as used in the present invention, do not include proteins that, 15 after release from a fusion protein and translocation into the nucleus, repress transcription from a reporter gene.

Among the transcription factors that are useful in the present invention are: HIV1 TAT (in particular exon I of HIV1 TAT), Gal4-VP16, the entire Gal4 protein, BIV TAT, HIV-2 TAT, SIV TAT, LexA-VP16, EBV Zta, Papillomavirus E2, 20 or one of the bHLH homodimeric transcription factors, E12, E47, or Twist.

Expression vectors are generally used to express the fusion protein in the recombinant cells. An expression vector contains recombinant nucleic acid encoding a polypeptide (e.g., an APP/transcription factor fusion protein) along with regulatory elements for proper transcription and processing. Generally, the regulatory 25 elements that are present in an expression vector include a transcriptional promoter, a ribosome binding site, a transcriptional terminator, and a polyadenylation signal. Other elements may include an origin of replication for autonomous replication in a host cell, a selectable marker, a limited number of useful restriction enzyme sites, and a potential for high copy number.

30 A variety of expression vectors are known in the art and can be used in the present invention. Commercially available expression vectors which are suitable include, but are not limited to, pMC1neo (Stratagene), pSG5 (Stratagene), pcDNAI and pcDNAIamp, pcDNA3, pcDNA3.1, pCR3.1 (Invitrogen, San Diego, CA), EBO- pSV2-neo (ATCC 37593), pBPV-1(8-2) (ATCC 37110), pdBPV-MMTneo(342-12)

(ATCC 37224), pRSVgpt (ATCC 37199), pRSVneo (ATCC 37198), pCI.neo (Promega), pTRE (Clontech, Palo Alto, CA), pV1Jneo, pIRESneo (Clontech, Palo Alto, CA), pCEP4 (Invitrogen, San Diego, CA), pSC11, and pSV2-dhfr (ATCC 37146). The choice of vector will depend upon the cell type in which it is desired to  
5 express the APP/transcription factor fusion protein, as well as on the level of expression desired, and the like.

The expression vectors can be used to transiently express or stably express the fusion protein. The transient expression or stable expression of transfected DNA is well known in the art. See, *e.g.*, Ausubel et al., 1995,  
10 "Introduction of DNA into mammalian cells," in Current Protocols in Molecular Biology, sections 9.5.1-9.5.6 (John Wiley & Sons, Inc.).

The recombinant host cells of the present invention are useful in methods of screening substances for the ability to inhibit APP processing. In one embodiment, the methods of the present invention comprise adding a candidate  
15 substance to a recombinant host cell comprising an APP/transcription factor fusion protein and a reporter gene and comparing the level of expression of the reporter gene protein in the presence and absence of the candidate substance, wherein the level of expression of the reporter gene protein is lower when the candidate substance inhibits processing of the APP/transcription factor fusion protein such that the transcription  
20 factor is not released, or is released in a lower amount, than in the absence of the substance.

The level of expression of the reporter gene protein is generally not measured directly. Rather, an indirect method is used. For example, fluorescence given off by the reporter gene protein may be detected or measured as, *e.g.*, when the  
25 reporter gene product is a green fluorescent protein; or, some enzymatic activity of the reporter gene product may be detected or measured, *e.g.*, when the reporter gene product is  $\beta$ -lactamase.

The candidate substance may be of any form suitable for entry into the cytoplasm of the recombinant cell or for contact with the cell's cytoplasmic  
30 membrane. Under appropriate conditions, the candidate substance may be allowed to freely diffuse into the cell, or the delivery of the substance may be facilitated by techniques and substances which enhance cell permeability, a wide variety of which are known in the art. Methods for increasing cell permeability include, without limitation, the use of organic solvents such as dimethylsulfoxide, liposomes,

application of electrical current, and physical means such as substance-coated teflon pellets.

The present invention provides a method of identifying a substance that inhibits APP processing comprising:

- 5 (a) providing a recombinant eukaryotic cell which:
  - (i) expresses a fusion protein comprising amino acids 589-651 of APP<sub>695</sub> and a transcription factor where the transcription factor is fused in frame to the carboxyl terminus of amino acids 589-651 of APP<sub>695</sub>; and
  - (ii) comprises a reporter gene operably linked to a regulatory
- 10 DNA sequence which is capable of being activated by the transcription factor;
- (b) measuring the level of reporter gene product in the cell in the absence of the substance;
- (c) adding the substance to the cell and measuring the level of reporter gene product in the cell in the presence of the substance;
- 15 where a decrease in the level of reporter gene product in the presence as compared to the absence of the substance indicates that the substance inhibits APP processing.

The manner in which the level of the reporter gene product is measured will be determined by the nature of the reporter gene and, often, the characteristics of the host cell. For example, if the reporter gene product itself is fluorescent, as for

20 example, when a green fluorescent protein is the reporter gene product, fluorescence from the cell can be measured directly. When the reporter gene product has enzymatic activity, for example, when the reporter gene product is  $\beta$ -lactamase, known methods of measuring that enzymatic activity can be used.

25 For the sake of clarity, the above method is described in terms of "a" cell. In actual practice, the method will generally be carried on a large number of cells at one time. For example, the method will often be carried out in a well of a tissue culture plate, where, depending on the number of wells in the plate (and thus their size), there can be up to hundreds, thousands, or even several million cells. The step

30 of "adding the substance to the cell" is generally carried out by simply adding the substance to the tissue culture medium in which the cells are present. After the substance is added to the cell, the cell and the substance are incubated for a period of time sufficient for the substance to inhibit APP processing, if the substance is actually

an inhibitor of APP processing. This period is usually from about 15 minutes to 48 hours, but may be somewhat more in unusual cases.

A convenient way of carrying out the method is to grow a population of the recombinant eukaryotic cells and then split the population into a portion that will be exposed to the substance and a portion that will not be exposed to the substance.

The recombinant eukaryotic cell is generally produced by transfection of an expression vector encoding the fusion protein and by transfection of a plasmid containing the reporter gene.

One skilled in the art would recognize that what is sought in terms of "a decrease in the level of reporter gene product in the presence as compared to the absence of the substance" is a non-trivial decrease. For example, if in the method described above there is found a 1% decrease, this would not indicate that the substance is an inhibitor of APP processing. Rather, one skilled in the art would attribute such a small decrease to normal experimental variance. What is looked for is a significant decrease. For the purposes of this invention, a significant decrease fulfills the usual requirements for a statistically valid measurement of a biological signal. For example, depending upon the details of the embodiment of the invention, a significant decrease might be a decrease of at least 10%, preferably at least 20%, more preferably at least 50%, and most preferably at least 90%.

In particular embodiments, amino acids 589-651 of APP<sub>695</sub> contain a K612V mutation.

In particular embodiments, the cell is a mammalian cell. In particular embodiments, the cell is a human cell.

In particular embodiments, the method is used to screen a library of more than 1,000 substances. In other embodiments, the method is used to screen a library of more than 50,000 substances at a rate of more than 1,000 substances per 24 hours.

In particular embodiments, the fusion protein comprises a portion of APP that is selected from the group consisting of: amino acids 1-651 of APP<sub>695</sub>, amino acids 50-651 of APP<sub>695</sub>, amino acids 100-651 of APP<sub>695</sub>, amino acids 150-651 of APP<sub>695</sub>, amino acids 200-651 of APP<sub>695</sub>, amino acids 250-651 of APP<sub>695</sub>, amino acids 300-651 of APP<sub>695</sub>, amino acids 350-651 of APP<sub>695</sub>, amino acids 400-

651 of APP<sub>695</sub>, amino acids 450-651 of APP<sub>695</sub>, amino acids 500-651 of APP<sub>695</sub>, and amino acids 550-651 of APP<sub>695</sub>.

In related embodiments, the fusion protein does not comprise all of amino acids 589-651 of APP<sub>695</sub>. Rather, the fusion protein comprises slightly fewer amino acids from APP. For example, the fusion protein might comprise slightly fewer amino acids of the  $\beta$ -secretase cleavage site: e.g., amino acids 590-651 of APP<sub>695</sub>. Or the fusion protein might comprise slightly fewer amino acids of the  $\gamma$ -secretase cleavage site: amino acids 589-650 of APP<sub>695</sub>; amino acids 589-649 of APP<sub>695</sub>; amino acids 589-648 of APP<sub>695</sub>; or amino acids 589-647. The fusion protein may even comprise slightly fewer amino acids from both ends, e.g., amino acids 590-647 of APP<sub>695</sub>. What is important is that the portion of APP included in the fusion protein contains both the  $\beta$ -secretase cleavage site and the  $\gamma$ -secretase cleavage site.

In particular embodiments, the transcription factor is selected from the group consisting of: HIV-1 TAT, Gal4-VP16, the entire Gal4 protein, LexA-VP16, EBV Zta, Papillomavirus E2, one of the bHLH homodimeric transcription factors, including E12, E47, or Twist, or BIV TAT, HIV-2 TAT, or SIV TAT. A particular version of HIV-1 TAT suitable for use in the present invention is HIV-1 TAT exon I.

Fusion proteins suitable for use in the present invention can be selected from the group consisting of: APP(1-651)SW, K612V-TATexonI(M1L) APP (664-695) (SEQ ID NO:2); APP(1-651)wt, K612V-TATexonI(M1L) APP (664-695) (SEQ ID NO:4); APP(1-651)SW, K612V, Gal4-VP16(M1L) APP (664-695) (SEQ ID NO:6); APP(1-651)wt, K612V, Gal4-VP16(M1L) APP (664-695) (SEQ ID NO:8); APP(1-651)SW, TATexonI(M1L) APP (664-695) (SEQ ID NO:10); APP(1-651)wt, TATexonI(M1L) APP (664-695) (SEQ ID NO:12); APP(1-651)SW, Gal4-VP16(M1L) APP (664-695) (SEQ ID NO:14); APP(1-651)wt, Gal4-VP16(M1L) APP (664-695) (SEQ ID NO:16); APP(1-651)NFEV, K612V-TATexonI(M1L) APP (664-695) (SEQ ID NO:23); and APP(1-651)NFEV, K612V, GAL4-VP16(M1L) APP (664-695) (SEQ ID NO:25).

In some embodiments of the present invention, the amino acid sequences contributed to the fusion protein by the transcription factor constitute the carboxy terminal amino acid sequences of the fusion protein. In other embodiments, the transcription factor has other sequences fused to its carboxy terminus, as in the examples herein where amino acids 664-695 of APP<sub>695</sub> are fused to the carboxy terminus of the transcription factor and therefore constitute the carboxy terminal

amino acid sequences of the fusion protein. Other portions of APP (e.g., amino acids 652-695 of APP<sub>695</sub>) could be used instead of amino acids 664-695 of APP<sub>695</sub>. In fact, it should be possible to extend the carboxy terminus of the transcription factor with almost any amino acid sequences, providing such sequences do not interfere with the ability of the transcription factor to move into the nucleus and activate transcription of the reporter gene once the transcription factor has been released from the fusion protein by the action of  $\gamma$ -secretase.

The present invention includes a method of identifying a substance that inhibits APP processing comprising:

- 10 (a) providing a recombinant eukaryotic cell which:
  - (i) expresses a fusion protein comprising an amino acid sequence from APP that is capable of being cleaved by both  $\beta$ -secretase and  $\gamma$ -secretase and a transcription factor where the transcription factor is fused in frame to the amino acid sequence from APP; and
  - 15 (ii) comprises a reporter gene operably linked to a regulatory DNA sequence which capable of being activated by the transcription factor;
- (b) measuring the level of reporter gene product in the cell in the absence of the substance;
- (c) adding the compound to the cell and measuring the level of  
20 reporter gene product in the cell in the presence of the substance;  
where a decrease in the level of reporter gene product in the presence as compared to the absence of the substance indicates that the substance inhibits APP processing.

In particular embodiments, the amino acid sequence from APP  
25 comprises:

- 589-651 of APP<sub>695</sub>;
- 590-651 of APP<sub>695</sub>;
- 589-650 of APP<sub>695</sub>;
- 590-650 of APP<sub>695</sub>;
- 30 589-649 of APP<sub>695</sub>;
- 590-649 of APP<sub>695</sub>;
- 589-648 of APP<sub>695</sub>;
- 590-648 of APP<sub>695</sub>;
- 589-647 of APP<sub>695</sub>; or

590-647 of APP<sub>695</sub>.

In related embodiments, the amino acid sequence from APP contains the amino acid sequence NLDA (SEQ ID NO:38) at the  $\beta$ -secretase cleavage site instead of the wild-type sequence KMDA (SEQ ID NO:34).

5 In related embodiments, the amino acid sequence from APP contains the amino acid sequence NFEV (SEQ ID NO:40) at the  $\beta$ -secretase cleavage site instead of the wild-type sequence KMDA (SEQ ID NO:34).

The portion of the fusion protein that is derived from APP may contain mutations that are known in the art. Of particular interest are mutations that result in an increased proportion of A $\beta$  being made in the form of A $\beta$ <sub>1-42</sub> rather than A $\beta$ <sub>1-40</sub>. Such mutations are disclosed in the following publications (numbering is from the 770 amino acid version of APP):

Swedish (K670N, M671L): Mullan et al., 1992, Nature Genet. 1:345-347.  
 Flemish (A692G): Hendriks et al., 1992, Nature Genet. 1:218-221; Cras et al., 1998,  
 15 Acta Neuropathol. (Berlin) 96:253-260.  
 Dutch (E693Q): Levy et al., 1990, Science 248:1124-1126.  
 Arctic (E693G): Nilsberth et al., 2001, Nature Neuroscience 4: 887-893.  
 Austrian (T714I): Kumar-Singh et al., 2000, Hum. Mol. Genet. 9:2589-2598.  
 French (V715M): Ancolio et al., 1999, Proc. Natl. Acad. Sci. (USA) 96:4119-4124.  
 20 Florida (I716V): Eckman et al., 1997, Hum. Mol. Genet. 6:2087-2089.  
 V717F: Murrell et al., 1991, Science 254:97-99.  
 V717G: Chartier-Harlin et al., 1991, Nature 353:844-846.  
 London (V717I): Goate et al., 1991, Nature 349:704-706.  
 L723P: Kwok et al., 2000, Ann. Neurol. 47:249-253.  
 25 I716F (also called I45F, referring to the position relative to the  $\beta$ -secretase cleavage site): This mutation in APP changes processing of A $\beta$  almost exclusively to A $\beta$ <sub>1-42</sub>.  
 Lichtenthaler et al., 1999, Proc. Natl. Acad. Sci. (USA) 96:3053-3058.

As with many proteins, it may be possible to modify many of the amino acids of the fusion proteins described above and still retain substantially the same biological activity in terms of APP processing as for the original fusion protein. Thus, the present invention includes modified fusion proteins which have amino acid deletions, additions, or substitutions but that still retain substantially the same properties with respect to APP processing as the fusion proteins described herein. It is generally accepted that single amino acid substitutions do not usually alter the



biological activity of a protein (see, *e.g.*, Molecular Biology of the Gene, Watson *et al.*, 1987, Fourth Ed., The Benjamin/Cummings Publishing Co., Inc., page 226; and Cunningham & Wells, 1989, Science 244:1081-1085). Accordingly, the present invention includes fusion proteins where one amino acid substitution has been made  
5 in the fusion proteins described herein where the fusion proteins still retain substantially the same properties with respect to APP processing as the fusion proteins described herein. The present invention also includes fusion proteins where two or more amino acid substitutions have been made in the fusion proteins described herein where the fusion proteins still retain substantially the same properties with respect to  
10 APP processing as the fusion proteins described herein. In particular, the present invention includes embodiments where the substitutions are conservative substitutions.

With the exception of Figure 18, the nucleotide and amino acid sequences of APP disclosed herein contain a minor difference compared to APP  
15 sequences that are usually reported in the literature. For the sequences disclosed herein with such a difference, the nucleotide at position 367 is an A rather than a G, as in most published APP sequences. This change results in a conservative substitution in the corresponding APP amino acid sequence. Thus, the amino acid sequences disclosed herein with such a difference have an I rather than a V at position 123. This  
20 difference does not affect the properties of the fusion proteins for the purposes of the present invention. Therefore, fusion proteins having the APP sequence reported in the literature with an G at nucleotide position 367 and a V at amino acid position 123 and the fusion proteins disclosed herein with an A at nucleotide position 367 and an I at amino acid position 123 are to be considered equivalents for the purposes of the  
25 present invention.

The Gal-VP16 sequences disclosed herein contain two changes from the usual published sequences. There is T to C change at nucleotide position 2131 that causes a S to P change at amino acid position 712; there is A to C change at nucleotide position 2301 that does not change the amino acid sequence. It is expected  
30 that Gal-VP16 proteins containing the usual sequences reported in the literature will also be suitable for use in the present invention.

The methods of the present invention can be used to screen libraries of substances or other sources of substances to identify substances that are inhibitors of  $\beta$ -secretase or  $\gamma$ -secretase. Such identified inhibitory substances can serve as "leads"

for the development of pharmaceuticals that can be used to treat patients having Alzheimer's disease or in a prophylactic manner to prevent or delay the development of Alzheimer's disease. Such leads can be further developed into pharmaceuticals by, for example, subjecting the leads to sequential modifications, molecular modeling, and other routine procedures employed in the pharmaceutical industry. The inhibitors of APP processing identified by the present invention may also be tested in animal models of Alzheimer's disease such as the various transgenic mouse models that are known in the art.

Although a wide variety of substances can be screened by the methods of the present invention, preferred substances for screening are libraries of small molecule compounds. Small molecule compounds are preferred because they are more readily absorbed after oral administration, have fewer potential antigenic determinants, and are more likely to cross the blood/brain barrier than larger molecules such as nucleic acids or proteins.

Once identified by the methods of the present invention, the candidate small molecule compounds may then be produced in quantities sufficient for pharmaceutical testing and formulated in a pharmaceutically acceptable carrier (see, e.g., Remington's Pharmaceutical Sciences, Gennaro, A., ed., Mack Publishing, 1990, for suitable methods). The candidate compounds may be administered to cell lines relevant to Alzheimer's disease, animal models of Alzheimer's disease, or Alzheimer's disease patients.

The numbering of the amino acids in APP used herein is based on the 695 amino acid version of APP described in Kang et al., 1987, Nature 325:733-736. There are two other major versions of APP, having 751 amino acids and 770 amino acids (see, Ponte et al., 1988, Nature 331:525-527 for the 751 amino acid version and Kitaguchi et al., 1988, Nature 331:530-532 for the 770 amino acid version). One skilled in the art will understand how to translate the numbering used herein, based on the 695 amino acid version of APP, into the corresponding numbering for other versions of APP. For example, some of the APP/transcription factor fusion proteins of the present invention contain the K612V mutation, based on the numbering of the 695 amino acid version. This mutation would correspond to a K668V mutation in the 751 amino acid version and a K687V mutation in the 770 amino acid version.

Therefore, when a "K612V" mutation is referred to herein, it will be understood that such reference also includes a K668V mutation of the 751 amino acid version of APP as well as a K687V mutation of the 770 amino acid version of APP.

Similarly, the portion of APP referred to as APP<sub>1-651</sub> herein, based on  
5 the 695 amino acid version, will be understood to mean also APP<sub>1-707</sub> of the 751 amino acid version and APP<sub>1-726</sub> of the 770 amino acid version.

If desired, inhibitors that are identified by the methods of the present invention can be further tested to determine which step in APP processing they affect. Assays that are known to be specific for the various steps of APP processing can be  
10 used for this purpose. For example, the assay of Karlström et al., (Journal of Biological Chemistry papers in press, published on December 13, 2001 as Manuscript C100649200) is only capable of detecting inhibitors of  $\gamma$ -secretase and cannot also detect inhibitors of other steps of APP processing such as, e.g., inhibitors of  $\beta$ -secretase. If an inhibitor identified by the methods of the present invention is found to  
15 also be an inhibitor when tested in the assay of Karlström et al., then that inhibitor is at least a  $\gamma$ -secretase inhibitor. It is still possible that that inhibitor could inhibit other steps in APP processing as well. Further tests known in the art can determine this.

The present invention may be modified so as to provide methods of determining at which step of APP processing a known inhibitor of APP processing  
20 exerts its effect. The known inhibitor may be one that has been identified by the methods of the present invention or by some other method. The modification to the present invention consists in mutating the  $\beta$ -secretase site in a fusion protein so that  $\beta$ -secretase cleavage can no longer occur at the site or occurs at a very much reduced level. Providing that the fusion protein contains a cleavable  $\alpha$ -secretase site, the  
25 fusion protein can still be used in the methods of the present invention. However, this fusion protein (with a mutated  $\beta$ -secretase site) can no longer detect  $\beta$ -secretase inhibitors. Therefore, if the known APP processing inhibitor still functions as an APP processing inhibitor in this modified version of the invention, then the known inhibitor cannot be a  $\beta$ -secretase site inhibitor but instead must exert its effect  
30 downstream of  $\beta$ -secretase.

Suitable mutations of the  $\beta$ -secretase site include the following. All the sequences are for amino acid positions 594-598 of APP<sub>695</sub>.

VNFAV (SEQ ID NO:41): This mutation shows decreased  $\beta$ -secretase cleavage relative to the wild type, KMDA (SEQ ID NO:34), sequence.

VKVDA (SEQ ID NO:42): Vassar et al., 1999, Science 286:735-741. This mutant was tested in vitro only, but purified  $\beta$ -secretase failed to cleave a 30-amino acid peptide containing this sequence.

- WKMDA (SEQ ID NO:43), VKADA (SEQ ID NO:44), VKKDA (SEQ ID NO:45),  
 5 VKEDA (SEQ ID NO:46), VKIDA (SEQ ID NO:47), VKMLA (SEQ ID NO:48),  
 VKMNA (SEQ ID NO:49), VKMEA (SEQ ID NO:50), VKMDE (SEQ ID NO:51),  
 VKMDK (SEQ ID NO:52): Citron et al., 1995. Neuron 14:661-670. These mutations decreased A $\beta$  production 4-20X relative to p3 production in cultured cells.

- Fusion proteins can be constructed by use of the polymerase chain  
 10 reaction (PCR) to amplify desired portions of APP and transcription factors, which can be then be cloned into expression vectors by methods well known in the art. Primers for PCR will generally include a small part of the APP or transcription factor as well as convenient cloning sites and/or linker peptide sequences. The PCR primers can be used to amplify the desired APP or transcription factor fragments from sources  
 15 such as previously cloned APP or transcription factors, cDNA libraries, or genomic libraries. The amplified APP and transcription factor sequences can be cloned into suitable expression vectors. Methods of PCR and cloning are well known in the art and can be found in standard reference materials such as those listed below.

- Standard techniques for cloning, DNA isolation, amplification and  
 20 purification, for enzymatic reactions involving DNA ligase, DNA polymerase, restriction endonucleases and the like, and various separation techniques are known and commonly employed by those skilled in the art. A number of standard techniques are described in Sambrook et al. (1989) Molecular Cloning, Second Edition, Cold Spring Harbor Laboratory, Plainview, N.Y.; Maniatis et al. (1982) Molecular Cloning,  
 25 Cold Spring Harbor Laboratory, Plainview, N. Y.; Wu (ed.) (1993) Meth. Enzymol. 218, Part I; Wu (ed.) (1979) Meth. Enzymol. 68; Wu et al. (eds.) (1983) Meth. Enzymol. 100 and 101; Grossman and Moldave (eds.) Meth. Enzymol. 65; Miller (ed.) (1972) Experiments in Molecular Genetics, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.; Old and Primrose (1981) Principles of Gene Manipulation,  
 30 University of California Press, Berkeley; Schleif and Wensink (1982) Practical Methods in Molecular Biology; Glover (ed.) (1985) DNA Cloning Vol. I and II, IRL Press, Oxford, UK; Hames and Higgins (eds.) (1985) Nucleic Acid Hybridization, IRL Press, Oxford, UK; Setlow and Hollaender (1979) Genetic Engineering: Principles

and Methods, Vols. 1-4, Plenum Press, New York, and Ausubel et al. (1992) Current Protocols in Molecular Biology, Greene/Wiley, New York, N.Y..

PCR reactions can be carried out with a variety of thermostable enzymes including but not limited to AmpliTaq, AmpliTaq Gold, or Vent polymerase.

- 5 For AmpliTaq, reactions can be carried out in 10 mM Tris-Cl, pH 8.3, 2.0 mM MgCl<sub>2</sub>, 200  $\mu$ M of each dNTP, 50 mM KCl, 0.2  $\mu$ M of each primer, 10 ng of DNA template, 0.05 units/ $\mu$ l of AmpliTaq. The reactions are heated at 95°C for 3 minutes and then cycled 35 times using suitable cycling parameters, including, but not limited to, 95°C, 20 seconds, 62°C, 20 seconds, 72°C, 3 minutes. In addition to these
- 10 conditions, a variety of suitable PCR protocols can be found in PCR Primer, A Laboratory Manual, edited by C.W. Dieffenbach and G.S. Dveksler, 1995, Cold Spring Harbor Laboratory Press; or PCR Protocols: A Guide to Methods and Applications, Michael et al., eds., 1990, Academic Press.

- It is desirable to sequence the DNA encoding the fusion proteins, or at
- 15 least the junction regions of the various portions (APP, transcription factor, linkers) of the fusion protein in order to verify that the desired portions have in fact been obtained, joined properly, and that no unexpected changes have been introduced into the sequences by the PCR reactions.

- Suitable PCR primers for amplification of DNA sequences for use in
- 20 the present invention can be readily designed by those of skill in the art. Examples of such primers are shown below.

5'-GGA GAG GAT ATC ATG GAG CCA GTA GAT CC-3' (SEQ ID NO:53) can be used to amplify the 5' portion of HIV-1 TAT exon I.

25

5'-TAC ATG GCG GCC GCC TAC TTA CTG CTT TG-3' (SEQ ID NO:54) can be used to amplify the 3' portion of HIV-1 TAT exon I.

- 5'-GGA TGT GAT ATC TTT CTT CTT CAG CAT CAC CAA GG-3' (SEQ ID
- 30 NO:55) can be used to amplify the 3' portion of DNA encoding amino acids 1-651 of APP, i.e., the transmembrane region of APP.

The following non-limiting examples are presented to better illustrate the invention.

## EXAMPLE 1

Transfection of pcDNA3.1 zeo (+) APP(1-651)SW, K612V-(M1L)TATexonI with  
pMM321

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5           The following example demonstrated that an APP/TAT fusion  
construct will transactivate a reporter gene in which the HIV1 LTR regulatory DNA  
sequence controls the expression of enhanced green fluorescent protein (EGFP). The  
following also serves as an example of the kind of preliminary routine variations of  
fusion protein levels and inhibitor levels that may be advantageous to test in the  
10 practice of the present invention. Such routine variations are often helpful in  
validating the assays before a large scale screening project is undertaken.

          The APP/TAT fusion construct is referred to as "pcDNA3.1 zeo (+)  
APP(1-651)SW, K612V-(M1L)TATexonI" (see Figure 26) and contains the HIV1  
TAT exon 1 fused just after the transmembrane domain of APP. This construct is also  
15 shown in outline form in Figure 1B. "pMM321" refers to a reporter gene plasmid  
consisting of the HIV1 LTR driving the transcription of enhanced green fluorescent  
protein (see Figure 25). As a positive control for TAT expression, a construct in  
which TAT was under the control of a strong, constitutive promoter (referred to as  
"pUCd5TAT"; see Figure 24) was used.

20

## METHODS:

1. Day 1: Pass HEK 293T cells into 2 x 6 well dishes at  $1 \times 10^5$  cells/well.
2. Day 2: Transfect cells with 9  $\mu$ L Fugene and 0.125  $\mu$ g pMM321 and various  
25 amounts of pcDNA3.1 zeo (+) APP(1-651)SW, K612V-(M1L)TATexonI.

- Plate 1:
1. 5  $\mu$ g pcDNA3.1 zeo (+) APP(1-651)SW, K612V-(M1L)TATexonI
  2. 2.5  $\mu$ g pcDNA3.1 zeo (+) APP(1-651)SW, K612V-  
(M1L)TATexonI
  - 30 3. 1.25  $\mu$ g pcDNA3.1 zeo (+) APP(1-651)SW, K612V-  
(M1L)TATexonI

4. 0.625 µg pcDNA3.1 zeo (+) APP(1-651)SW, K612V-(M1L)TATexonI
5. 0.312 µg pcDNA3.1 zeo (+) APP(1-651)SW, K612V-(M1L)TATexonI
- 5 6. 0.156 µg pcDNA3.1 zeo (+) APP(1-651)SW, K612V-(M1L)TATexonI
- Plate 2:
1. 0.08 µg pcDNA3.1 zeo (+) APP(1-651)SW, K612V-(M1L)TATexonI
2. 0.04 µg pcDNA3.1 zeo (+) APP(1-651)SW, K612V-(M1L)TATexonI
- 10 3. no pcDNA3.1 zeo (+) APP(1-651)SW, K612V-(M1L)TATexonI
4. 0.625 pUCd5TAT
5. 0.625 µg pcDNA3.1 zeo (+) APP(1-651)SW, K612V-(M1L)TATexonI and 1 µg pMM321
- 15 6. 1 µg pMM321

Six hours post-transfection, green cells were only observed in plate 2, #5.

3. Day 3: The fluorescence intensity of the transfected cells was observed and recorded.
- 20
4. Day 4: The fluorescence intensity of transfected cells was observed and recorded.

#### RESULTS:

- 25 Co-transfection with pcDNA3.1 zeo (+) APP(1-651)SW, K612V-(M1L)TATexonI increased GFP expression in the cells.

Day 3:

- 5 µg – no green cells
- 30 • 2.5 µg – no green cells (too much DNA for these two transfections?)
- 1.25 µg - many bright and dim green cells (see photographs and figure in ancillary data)
- 0.625 µg - bright and dim green cells but fewer than at 1.25 µg
- 0.312 µg - no difference obvious between 0.625 µg and 0.312 µg

- 0.156  $\mu$ g - very few green cells
- 0.08  $\mu$ g - very few green cells
- 0.04  $\mu$ g - very few green cells
- no pcDNA3.1 zeo (+) APP(1-651)SW, K612V-(M1L)TATexonI - extremely few  
5 (if any) green cells
- 0.625  $\mu$ g pUCd5TAT - cells were extremely bright, not necessarily more in  
number than with pcDNA3.1 zeo (+) APP(1-651)SW, K612V-(M1L)TATexonI
- 0.625  $\mu$ g pcDNA3.1 zeo (+) APP(1-651)SW, K612V-(M1L)TATexonI + 1  $\mu$ g  
pMM321 - many bright and dim green cells.
- 10 • 1  $\mu$ g pMM321 many-fold fewer green cells, some bright, most dim.

Day 3 - changed media (saved 1 mL conditioned media from wells Plate 1- 3, 4, 5, 6;  
Plate 2 - 1, 2, 3, 5, 6). Added fresh media with 10  $\mu$ M of L-685,458 (a potent, cell  
permeable  $\gamma$ - secretase inhibitor) to wells Plate 1 - 3, 4, 5, 6; Plate 2 - 3, 4, 5, 6.  
15 Waited 48 hours to observe loss of fluorescence since GFP is so stable.

After 48 hours, all wells appeared brighter than at 24 hour time point. This does not  
necessarily mean that the inhibitor was ineffective, or that the assay did not work,  
since there were no controls run where the inhibitor was not added. However, it does  
20 suggest that under these conditions it may be preferable to add the inhibitor at the time  
of transfection to shut down  $\gamma$ -secretase as soon as possible and avoid release of TAT  
and induction of GFP.

## EXAMPLE 2

- 25 Transfection of APP(1-651)SW, K612V-(M1L)TATexonI into HEK293T and H4  
cells accompanied by inhibition of  $\gamma$ -secretase activity with L-685,458

The following example demonstrates the operation of the invention in  
HEK293T cells and H4 cells and shows inhibition of APP processing (and thus TAT  
release) by treatment with a known  $\gamma$ -secretase inhibitor. "pcDNA3.1 zeo (+) APP(1-  
30 651)SW, K612V-(M1L)TATexonI," "pMM321," and "pUCd5TAT" are the same as  
in Example 1. H4 cells (ATCC HTB-148) are a neuronal cell line.



## METHODS:

Day1: Plated out 2 x 6 well plates of HEK293T cells and 2 x 6 well plates of H4 cells at  $1 \times 10^5$  cells/well.

- 5 Day 2: Transfected cells with 2  $\mu$ g total DNA - pcDNA3.1 zeo (+) APP(1-651)SW, K612V-(M1L)TATexonI and carrier (a pET-IN plasmid).

Plate1:

- 1,2: 1  $\mu$ g pMM321 + 1  $\mu$ g pcDNA3.1 zeo (+) APP(1-651)SW, K612V-(M1L)TATexonI
- 10 3,4: 1  $\mu$ g pMM321 + 0.1  $\mu$ g pcDNA3.1 zeo (+) APP(1-651)SW, K612V-(M1L)TATexonI + 0.9  $\mu$ g carrier
- 5: 1  $\mu$ g pMM321 + 1  $\mu$ g carrier (added too much carrier to this well in H4 cells)
- 6: 1  $\mu$ g pMM321 + 0.1  $\mu$ g pUCd5TAT + 0.9  $\mu$ g carrier

- 15 Plate 2:

- 1,2: 0.1  $\mu$ g pMM321 + 1  $\mu$ g pcDNA3.1 zeo (+) APP(1-651)SW, K612V-(M1L)TATexonI + 0.9  $\mu$ g carrier
- 3,4: 0.1  $\mu$ g pMM321 + 0.1  $\mu$ g pcDNA3.1 zeo (+) APP(1-651)SW, K612V-(M1L)TATexonI +
- 20 1.8  $\mu$ g carrier (added too 0.5X carrier to this mix in 293T cells)
- 5: 0.1  $\mu$ g pMM321 + 1.9  $\mu$ g carrier
- 6: 0.1  $\mu$ g pMM321 + 0.1  $\mu$ g pUCd5TAT + 1.8  $\mu$ g carrier

- Transfections for HEK293T cells: 9  $\mu$ L Fugene/well. Combined with DNA in
- 25 Optimem and incubated and added to cells according to manufacturer's instructions.

Transfections for H4 cells: 6  $\mu$ L Fugene/well. Combined with DNA in Optimem and incubated and added to cells according to manufacturer's instructions.

- 30 Added 10  $\mu$ M L-685,458 to Plates 1 and 2, wells 2 and 4 for both cell types within 1 hour of transfection. Observed cells periodically.

Took pictures at 24, 46 hours after transfection, using AE lock to keep exposures constant between wells.

## RESULTS:

Both H4 and 293T cells turned much brighter green in the presence of pcDNA3.1 zeo (+) APP(1-651)SW, K612V-(M1L)TATexonI

5 At 24 hours:

H4 cells:

Plate 1: 1 ug pMM321

1. 1 ug pMM321 + 1 ug pcDNA3.1 zeo (+) APP(1-651)SW, K612V-(M1L)TATexonI: Many  
10 bright and dim green cells (good transfection efficiency as well)
  2. 1 ug pMM321 + 1 ug pcDNA3.1 zeo (+) APP(1-651)SW, K612V-(M1L)TATexonI + 10  $\mu$ M L-685,458: Also many bright and dim green cells, but reduced compared with  
well #1
  - 15 3. Very few green cells (a few per field)
  4. Very few green cells
  5. A few dimly green cells
  6. Some induction with 0.1  $\mu$ g pUCd5TAT but still relatively few cells.
- 20 Plate 2: 0.1  $\mu$ g pMM321
1. 0.1  $\mu$ g pMM321 + 1  $\mu$ g pcDNA3.1 zeo (+) APP(1-651)SW, K612V-(M1L)TATexonI:  
~10 bright green cells/field and the rest are dim green
  2. 0.1  $\mu$ g pMM321 + 1  $\mu$ g pcDNA3.1 zeo (+) APP(1-651)SW, K612V-(M1L)TATexonI + 10  $\mu$ M L-685,458: ~3-5 bright green cells/field, some dim green,  
25 and some not green.
  3. No green cells
  4. No green cells
  5. No green cells
  - 30 6. A few bright green cells

HEK293T cells:

Plate 1: 1  $\mu$ g pMM321

1. + 1 µg pcDNA3.1 zeo (+) APP(1-651)SW, K612V-(M1L)TATexonI: of 15 cells:  
6 dim, 4  
medium, 5 bright
2. + 1 µg pcDNA3.1 zeo (+) APP(1-651)SW, K612V-(M1L)TATexonI + 10 µM L-  
5 685,458:  
of 15 cells: 6 very dim, 5 dim, 1 medium, 3 bright
3. +0.1 µg pcDNA3.1 zeo (+) APP(1-651)SW, K612V-(M1L)TATexonI: many more  
green  
cells than 1 µg, lots of strong, bright green cells
- 10 4. + 0.1 µg pcDNA3.1 zeo (+) APP(1-651)SW, K612V-(M1L)TATexonI + 10 µM L-  
685,458:  
Fewer bright green cells/field but intensity does not appear strongly diminished
5. 1 µg pMM321 alone: Most cells in the field expressing dim to medium levels of  
GFP
- 15 6. enhancement by 0.1 µg pUCd5TAT

Plate 2: 0.1 µg pMM321

1. + 1 µg pcDNA3.1 zeo (+) APP(1-651)SW, K612V-(M1L)TATexonI: Bright and  
medium  
20 green cells
2. + 1 µg pcDNA3.1 zeo (+) APP(1-651)SW, K612V-(M1L)TATexonI + 10 µM L-  
685,458:  
Bright, medium, and dim green cells
3. + 0.1 µg pcDNA3.1 zeo (+) APP(1-651)SW, K612V-(M1L)TATexonI: Bright,  
25 medium,  
and dim green cells
4. + 0.1 µg pcDNA3.1 zeo (+) APP(1-651)SW, K612V-(M1L)TATexonI + 10 µM L-  
685,458:  
Bright, medium, and dim green cells
- 30 5. 0.1 µg pMM321 alone: Most expressing cells have dim GFP, a few medium to  
bright  
cells
6. Enhancement by 0.1 µg pUCd5TAT

Changed media on cells at 24 hours past transfection. Kept 10  $\mu$ M L-685,458 on cells in wells 2 and 4.

At 46 hours after transfection, examined the wells again. Lots of floating cells in all wells, all cell types. Highest number of floaters in 1  $\mu$ g pcDNA3.1 zeo (+) APP(1-651)SW, K612V-(M1L)TATexonI lanes.

Took some photographs under fluorescent and white light (white light at low intensity) to reveal fluorescent and non-fluorescent cells. Conducted a subjective analysis of the photographs to see if the amount of inhibition by 10  $\mu$ M L-685,458 was in any way quantifiable. Counted bright (white in the middle); strong (blue middle), medium (green) and dim/non-fluorescent cells and determined the approximate fraction of each level of expression. Results follow:

15

TABLE 1

293T cells transfected with X  $\mu$ g pMM321 (first number in left column) and X  $\mu$ g pcDNA3.1 zeo (+) APP(1-651)SW, K612V-(M1L)TATexonI (second number in left column)

Transfection	# Bright	# Strong	# Med	# Non	% Bright	%Strong	% Med	%Non
1 $\mu$ g + 1 $\mu$ g	none	8	81	316		2	20	78
1 + 1 + cmpd	none	5	66	521		0.8	11	88
1 + 0.1	41	77	143	61	12	24	44	19
1+ 0.1+cmpd	23	32	149	194				

20 The results shown in Table 1 indicate that the presence of L-685,458 ("cmpd") caused fewer strong and medium fluorescing cells as well as more non-fluorescent cells in the first run; in the second run, L-685,458 caused fewer bright and strong fluorescing cells as well as more non-fluorescent cells (with slightly more medium fluorescing cells). Overall, these data clearly indicate that the presence of an inhibitor of APP processing  
25 such as L-685,458 can be identified by the present invention.

1 mL of conditioned media from each well was analyzed for production of A $\beta$ . Higher than background levels of A $\beta$  were observed in 293T cells after transfection

with 1  $\mu$ g pcDNA3.1 zeo (+) APP(1-651)SW, K612V-(M1L)TATexonI and 0.1  $\mu$ g pcDNA3.1 zeo (+) APP(1-651)SW, K612V-(M1L)TATexonI and higher than background levels of A $\beta$  in H4 cells after transfection with 1  $\mu$ g pcDNA3.1 zeo (+) APP(1-651)SW, K612V-(M1L)TATexonI, but not 0.1  $\mu$ g pcDNA3.1 zeo (+) APP(1-651)SW, K612V-(M1L)TATexonI (no enhancement of GFP was observed with 0.1  $\mu$ g pcDNA3.1 zeo (+) APP(1-651)SW, K612V-(M1L)TATexonI either). A $\beta$  was completely inhibited to background levels by 10  $\mu$ M L-685,458. Surprisingly, substantial inhibition of GFP was not observed with 10  $\mu$ M L-685,458.

10 100,000 cells from each well were trypsinized and placed in 0.1 mL phenol red-free media in a Costar 96-well dish and read using the fluorometer. The results are shown below:

TABLE 2

A			1 $\mu$ gAPPTAT	10 $\mu$ M 458	0.1 $\mu$ gAPPtat	10 $\mu$ M458	no tat	0.1 $\mu$ g pUCd5STAT				
B	293T	1 $\mu$ g LTRGFP	17670	14321	65535	65535	14890	65535				
C		0.1 $\mu$ g LTRGFP	9976	10491	17677	14790	9624	25735				
D	H4	1 $\mu$ g LTRGFP	21307	25307	7175	7136	7147	7277				
E		0.1 $\mu$ g LTRGFP	9574	10031	7317	6957	6946	7247				
F		Blank	7498	7124	7570	7454	5774	7638				
G												
H												
	1	2	3	4	5	6	7	8	9	10	11	12

15 In Table 2, "APPtat" is pcDNA3.1 zeo (+) APP(1-651)SW, K612V-(M1L)TATexonI. "458" is L-685,458. "LTRGFP" is pMM321.

0.1 µg pcDNA3.1 zeo (+) APP(1-651)SW, K612V-(M1L)TATexonI with and without compound exceeded the maximum reading of the fluorometer, as did the addition of 0.1 µg pUCd5TAT to cells transfected with 1 µg pMM321.

1 µg pcDNA3.1 zeo (+) APP(1-651)SW, K612V-(M1L)TATexonI + 1 µg pMM321  
5 incrementally increased the amount of fluorescence relative to 1 µg pMM321 alone, and this was reduced to background levels by 10 µM L-685,458. Inhibition of fluorescence was also observed in 293T cells transfected with 0.1 µg pcDNA3.1 zeo (+) APP(1-651)SW, K612V-(M1L)TATexonI + 0.1 µg pMM321. No inhibition of fluorescence was observed in H4 cells under any transfection conditions.

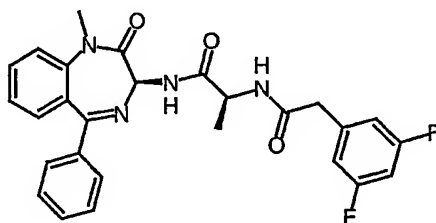
10

### EXAMPLE 3

#### Use of APP(1-651)SW, K612V-TATexonI in H4 cells

L-875,532 is a known γ-secretase inhibitor having the structure shown below. It is described and details of its synthesis are disclosed in Seiffert et al., 2000, J. Biol.

15 Chem. 275:34086-34091.



L-875532

Compound X is a β-secretase inhibitor.

20

pRBR186 (Figure 22A) is an expression vector containing DNA sequences encoding full-length APP containing the Swedish mutation and the K612V mutation. pRBR186 does not contain a transcription factor fused to the APP sequences.

pcDNA3.1 zeo (+) APP(1-651)SW, K612V-(M1L)TATexonI is an expression vector that directs the expression of a the fusion protein APP(1-651)SW, K612V-(M1L)TATexonI in mammalian cells. This fusion protein contains the first 651 amino acids of APP (with a Swedish version of the  $\beta$ -secretase cleavage site as well as the K612V mutation) fused in frame to exon I of HIV1 TAT, which has been modified with a Met1-Leu mutation. A schematic diagram of pcDNA3.1 zeo (+) APP(1-651)SW, K612V-(M1L)TATexonI is shown in Figure 26A. The nucleotide sequence of pcDNA3.1 zeo (+) APP(1-651)SW, K612V-(M1L)TATexonI (SEQ ID NO:22) is shown in Figure 26B-G.

10

## METHODS:

1. H4 cells (ATCC HTB-148) were transfected with the various constructs listed below using 6  $\mu$ L Fugene per 100  $\mu$ L Optimem and 100  $\mu$ L Optimem per well (6-well dishes). Transfection reactions were incubated for 30 minutes prior to adding 100  $\mu$ L dropwise onto wells.

15

Transfections were done as follows:

1. 1  $\mu$ g pMM321 (Figure 25A-D) and 1  $\mu$ g pcDNA3.1 backbone
- 20 1a. 1  $\mu$ g pMM321 and 1  $\mu$ g pcDNA3.1 (Invitrogen, San Diego, CA) backbone. Prior to transfection, 10  $\mu$ M L-875,532 ( $\gamma$ -secretase inhibitor) was added to the well.
2. 1  $\mu$ g pMM321 and 1  $\mu$ g pRBR186 (Figure 22A; APP expression vector; processing and inhibition of processing control)
- 25 2a. 1  $\mu$ g pMM321 and 1  $\mu$ g pRBR186. Prior to transfection, 10  $\mu$ M L-875,532 was added to cells (transfection solution for 3-5 were prepared in bulk)
3. and 3a. 1  $\mu$ g pMM321 and 1  $\mu$ g pcDNA3.1 zeo (+) APP(1-651)SW, K612V-(M1L)TATexonI
- 30 4. and 4a. 1  $\mu$ g pMM321 and 1  $\mu$ g pcDNA3.1 zeo (+) APP(1-651)SW, K612V-(M1L)TATexonI. Prior to transfection, 10  $\mu$ M L-875,532 was added to the two wells.
5. and 5a. 1  $\mu$ g pMM321 and 1  $\mu$ g pcDNA3.1 zeo (+) APP(1-651)SW, K612V-

(M1L)TATexonI. Prior to transfection, 10  $\mu$ M Compound X was added to the two wells

6. 1  $\mu$ g pMM321 and 1  $\mu$ g pUCd5TAT (Figure 24).

7. 1  $\mu$ g pMM321 and 1  $\mu$ g pUCd5 TAT. Prior to transfection, 10  $\mu$ M L-875,532 was added to the cells.

#### RESULTS:

Cells were assessed by eye under a fluorescence microscope the morning following transfection (~20 hrs).

1 and 1a, 2 and 2a. Weak fluorescence

3 and 3a. Much stronger fluorescence

4 and 4a. Clear inhibition of fluorescence

5 and 5a. Possible inhibition of fluorescence, but doesn't look that great

6 and 7. Almost blindingly fluorescent.

At approximately 48 hours, cells were trypsinized, spun down, and resuspended in 100  $\mu$ L PBS. The cellular contents of each well of the transfection plates were placed into one well of a 96-well fluorescence plate. Fluorescence was analyzed using the FLUOstar (485 excitation/538 emission). The results are shown in Table 3.

TABLE 3

Transient transfections in H4 cells	Fluor Units
pMM321	4484
pMM321 + L-875,532	3443
pMM321 + pRBR186	2735
pMM321 + pRBR186 + L-875,532	2161
pMM321 + APP-TAT-ct32	20177
pMM321 + APP-TAT-ct32 + L-875,532	8283
pMM321 + APP-TAT-ct32 + Compound X	11946
pMM321 + pucd5-TAT	61102

In Table 3, "APP-TAT-ct32" refers to pcDNA3.1 zeo (+) APP(1-651)SW, K612V-(M1L)TATexonI.



For a graphical presentation of these results, see Figure 19. In Figure 19, "LTR-GFP" refers to pMM321; "APP-TAT-ct32" refers to pcDNA3.1 zeo (+) APP(1-651)SW, K612V-(M1L)TATexonI. Compare the bar labeled "LTR-GFP + APP-TAT-ct32" with the bars labeled "LTR-GFP + APP-TAT-ct32 + L-875,532" and "LTR-GFP + APP-TAT-ct32 + Compound X." Inhibition by both the  $\beta$ -secretase inhibitor (Compound X) and the  $\gamma$ -secretase inhibitor (L-875,532) is easily identified by the present invention.

10 Conclusions:

- The data indicate that the expression of the fusion protein APP(1-651)SW, K612V-(M1L)TATexonI enhances transactivation through the LTR of pMM321 in a manner that depends on APP processing.
- APP(1-651)SW, K612V-(M1L)TATexonI expressing cells were 6X brighter than pMM321 cells alone.
- Treatment with L-875,532 decreased fluorescence 2.5X.
- Treatment with Compound X decreased fluorescence 1.7X.
- Expression of TAT via pucd5-TAT was almost blinding and was 19X above pMM321 alone, indicating that APP(1-651)SW, K612V-(M1L)TATexonI expression did not lead to levels of GFP as high as TAT alone. Despite the decreased activation shown by TAT when provided by the fusion protein, as compared with TAT driven by the AMLP (adenovirus major late promoter) in pucd5-TAT, the assay was easily able to identify both the  $\beta$ -secretase and the  $\gamma$ -secretase inhibitors.
- Control plasmids (pMM321 and pMM321 + pRBR186) were dimly fluorescent and were not inhibited by L-875,532.

A lower level of inhibition by the  $\beta$ -secretase inhibitor is to be expected since the K612V mutation decreases alpha-secretase activity by 95% and thus some alpha-secretase cleavage is to be expected.

30

## EXAMPLE 4

Construction of pcDNA3.1 (+) zeo APP(1-651)SW, K612V, GAL4-VP16(M1L) APP  
(664-695)

---

1. The GAL4-VP16 insert was prepared by PCR from pCR2.1 GAL4VP16 (Figure  
5 20) (Invitrogen, San Diego, CA). The PCR was performed to eliminate the N-  
terminal methionine by changing this methionine into a leucine.

- 40 ng pCR2.1 GAL4-VP16  
0.2  $\mu$ L GAL4VP16 5' oligo at 250  $\mu$ M:  
10 5'-CTGAGATATCAAGCTACTGTCTTCTATCGAACAAGC-3' (SEQ ID NO:56)  
EcoRV site underlined  
0.2  $\mu$ L GAL4VP16 3' oligo (at 250  $\mu$ M): 5'-  
GCGCGATATCCCCACCGTACTCGTCAATTCC-3' (SEQ ID NO:57)  
EcoRV site underlined  
15 5  $\mu$ L 10X Buffer  
8  $\mu$ L 25 mM MgCl<sub>2</sub>  
4  $\mu$ L PCR dNTPs  
0.25  $\mu$ L AmpliTaq Gold  
27.35  $\mu$ L water

20

Cycle:

- Purified reactions using a Qiaquick column  
Digested entire reaction using EcoRV  
25 Ran the DNA on a 1% gel. Excised the band and purified using a QiaQuick gel  
purification kit

2. Digested pcDNA3.1 APP(1-651)/APP(664-695) with EcoRV and SAP treated.  
pcDNA3.1 APP(1-651)/APP(664-695) is an intermediate plasmid formed in the  
30 procedure described in Example 6. pcDNA3.1 APP(1-651)/APP(664-695) the first  
651 amino acids of APP (with a Swedish version of the  $\beta$ -secretase cleavage site as  
well as the K612V mutation) fused in frame to the last 32 amino acids of APP.

3. Ligated pcDNA3.1 APP(1-651)/APP(664-695) –EcoRV digested to GAL4VP16 (EcoRV digested)

- 5     4. At this point, it was realized that the 3' PCR primer for GAL4-VP16 put the APP(664-695) fragment out of frame. The APP(664-695) fragment was then re-PCR'd using the following protocol:

- 1 µL pcDNA3.1 APP(1-651)-Gal4VP16-APP(664-695)  
10    50 nM APP NotI 5'ct32 in frame with GAL4-VP16  
50 nM APP Noti 3' ct32 (5'  
(p)CTGCTGTGGCGGCCGCTAGTTCTGCATCTGCTC) (SEQ ID NO:58)  
NotI site underlined  
1 µL PCR dNTPs (10 mM each dNTP, Roche)  
15    5 µL 10X Expand Buffer with MgCl<sub>2</sub>  
40 µL water  
1 µL Expand polymerase (Roche)

- The PCR fragment was run on a 4% agarose gel and gel-purified using a QiaQuick gel  
20    purification column  
The fragment was digested with NotI and purified using a QiaQuick PCR purification column.

5. APP(1-651)-Gal4VP16-APP(664-695) was re-miniprepmed. Miniprep #1 was  
25    digested with NotI, run on a 1% gel, the upper band was then isolated and SAP-treated.

6. APP(1-651)-Gal4VP16/NotI digested/SAP-treated was ligated to APP(664-695).

- 30    7. Minipreps containing inserts were sequenced to verify the orientation of the insert.

## EXAMPLE 5

Construction of pcDNA3.1 zeo (+) APP(1-651)wt, K612V-TATexonI(M1L)  
APP(664-695)

---

5 This procedure replaced a fragment of APP in pcDNA3.1 zeo (+) APP(1-651)SW,  
K612V-TATexonI(M1L) APP (664-695) that contained the Swedish mutation with a  
corresponding fragment from pRBR121 containing the wild-type  $\beta$ -secretase cleavage  
site rather than the Swedish  $\beta$ -secretase cleavage site.

1. pcDNA3.1 zeo (+) APP(1-651)SW, K612V-TATexonI(M1L) APP (664-695)  
10 (Figure 21B) was digested with SnaBI and then EcoRI

17  $\mu$ L miniprep DNA

2  $\mu$ L 10X buffer

1  $\mu$ L SnaBI (NEB)

- 15 Digest was purified using Qiaquick PCR purification kit. Entire digest was then  
cleaved with EcoRI for 2 hours.

2. pRBR121 (Figure 21A) was digested with SnaBI and then EcoRI

5  $\mu$ g pRBR121

20 5  $\mu$ L 10X buffer

2.5  $\mu$ L SnaBI (NEB)

q.s. 50  $\mu$ L with water

- 25 The digest was purified using a Qiaquick PCR purification kit. The entire digest was  
then cleaved with EcoRI for 2 hours.

3. Both digests were run out on a 1% agarose gel. From the pRBR121 lane, the 2.4  
kb SnaBI-EcoRI fragment containing the wild-type  $\beta$ -secretase cleavage site was  
isolated.

30

From pcDNA3.1 zeo (+) APP(1-651)SW, K612V-TATexonI(M1L) APP (664-695) digest, BOTH the 5 kb SnaBI-EcoRI backbone fragment AND the 200 bp EcoRI-EcoRI fragment were isolated (see Figure 21B).

- 5     4. A three-part ligation using equal molar ratios of the three fragments was carried out:  
The assumption was made that, since the starting plasmids were of similar sizes and the same amount was digested for each plasmid, the recovered fragments would be in approximately equal molar ratios.

10

Vector alone:

1  $\mu$ L APP-TAT-ct32 SnaBI/EcoRI 5Kb fragment

7  $\mu$ L water

2  $\mu$ L 5X buffer

- 15     10  $\mu$ L 2X buffer (Roche rapid ligation kit)

1  $\mu$ L T4 ligase

1+1+1

1  $\mu$ L pcDNA3.1 zeo (+) APP(1-651)SW, K612V-TATexonI(M1L) APP (664-695)

- 20     SnaBI/EcoRI 5KB fragment

1  $\mu$ L pRBR121 SnaBI/EcoRI 2.4 Kb insert

1  $\mu$ L pcDNA3.1 zeo (+) APP(1-651)SW, K612V-TATexonI(M1L) APP (664-695)

EcoRI/EcoRI 200 bp insert

5  $\mu$ L water

- 25     2  $\mu$ L 5X buffer

10  $\mu$ L 2X buffer

1  $\mu$ L T4 ligase

- 30     1+1+...1 (in this 3-pt ligation, the ligation of two of the fragments together was done 1<sup>st</sup>, then the third fragment was added)

1  $\mu$ L pcDNA3.1 zeo (+) APP(1-651)SW, K612V-TATexonI(M1L) APP (664-695)

SnaBI/EcoRI 5Kb backbone

1  $\mu$ L pcDNA3.1 zeo (+) APP(1-651)SW, K612V-TATexonI(M1L) APP (664-695)

EcoRI/EcoRI 200 bp insert

- 5  $\mu$ L water  
2  $\mu$ L 5X buffer  
10  $\mu$ L 2X buffer  
1  $\mu$ L T4 ligase  
5 waited 5 minutes  
then added 1  $\mu$ L pRBR121 SnaBI/EcoRI 2.4 kb insert
- 1+1+3  
1  $\mu$ L pcDNA3.1 zeo (+) APP(1-651)SW, K612V-TATexonI(M1L) APP (664-695)  
10 SnaBI/EcoRI 5 kb backbone  
1  $\mu$ L pRBR 121 SnaBI/EcoRI 2.4 kb insert  
3  $\mu$ L pcDNA3.1 zeo (+) APP(1-651)SW, K612V-TATexonI(M1L) APP (664-695)  
EcoRI/EcoRI 200 bp insert  
3  $\mu$ L water  
15 2  $\mu$ L 5X buffer  
10  $\mu$ L 2X buffer  
1  $\mu$ L T4 ligase
- Transformed and plated out 200  $\mu$ L. The number of colonies in the vector + insert  
20 ligations far exceeded the number of colonies in the vector alone ligation. Picked 12 colonies from 1+ 1+...1.  
Picked 6 colonies from 1+3.  
Miniprepped  
Digested with EcoRI to ensure that small 200 bp fragment was incorporated.  
25 RESULTS  
Minipreps #10 and 15 contained 200 bp EcoRI fragment.
- Oriented with Bam HI digestion.
- 30 Sequenced with sAPPb F2 and F3 primers. Miniprep #15 contains both inserts in the correct orientation.

## EXAMPLE 6

Construction of pcDNA3.1 zeo (+) APP(1-651)SW, K612V-TATexonI(M1L)  
APP(664-695)

---

1. PCR of APP (664-695):

5 The starting material was the pRBR186 plasmid (Figure 22A).

PCR of APP (664-695)

1 ng pRBR186

50 nM 5' oligo (5'-TGCCCCGCGCGGCCGCGCGATGCTGCCCCGG-3') (SEQ ID

10 NO:59) NotI site underlined

50 nM 3' oligo (5'-(p)ATGGTGTGGCGGCCGCAGACGCCGCTGTCACC-3')

(SEQ ID NO:60) NotI site underlined

1 µL Roche PCR nucleotides

5 µL 10X Expand buffer

15 40 µL water

1 µL Expand

Cycle: (94°C, 5 min) – 25X (94°C, 30 sec; 42°C, 1 min; 72°C, 2 min) – 72°C x 6 min  
– 4°C hold.

20

The ~100 bp fragment was gel purified (4% agarose, 1X TBE gel)

The gel-purified fragment was ligated into NotI digested, Shrimp Alkaline  
Phosphatase-treated pcDNA3.1 zeo (+) (Invitrogen). The presence of the insert and  
25 its orientation was confirmed by sequencing.

2. PCR of APP(1-651):

1 ng pRBR186

50 nM 5' oligo (5'-(p)AGCGCACAAAGCTTCCCCGCGCAGGGTCGCGATGCTG-  
30 3') (SEQ ID NO:61) HindIII site underlined, Met(1) ATG of APP in bold

50 nM 3' oligo (5'-(p)GGATGTAAGCTTTTTTCTTCTTCAGCATCACCAAGG-3')

(SEQ ID NO:62) HindIII site is underlined

- 1  $\mu$ L Roche PCR nucleotides  
 5  $\mu$ L 10X Expand buffer  
 40  $\mu$ L water  
 1  $\mu$ L Expand
- 5
- Cycle: (94°C, 5 min) -- 25X (94°C, 30 sec; 37°C, 1 min; 72°C 2.5 min) -- 72°C x 6 min -- 4°C hold
- 10
- The amplified fragment was isolated on an agarose gel. The fragment was purified from the gel using Qiaquick Gel purification columns. The fragment was digested with HindIII. The amount of the fragment was too small to subclone, so the PCR was repeated using 1  $\mu$ L of the amplified fragment and carrying out 5 reactions simultaneously.
- 15
- The fragments were purified from these reactions using a QiaQuick PCR purification kit. The fragments were eluted in 30  $\mu$ L and digested with HindIII for 2 hours. The digested fragments were then gel purified.
- 20
- The purified fragments were ligated to pcDNA3.1 zeo (+) APP(664-695) that had been digested with HindIII and SAP treated. This gave the intermediate plasmid pcDNA3.1 zeo (+) APP(1-651)/APP(664-695).
3. PCR of (M1L) TAT:  
 The starting material was NL4-3 viral plasmid (Figure 22B).
- 25
- PCR reaction:  
 1 ng NL4-3 viral plasmid  
 50 nM TAT 5' RV Met-Leu PCR primer (5'-  
TGCAGATATCCTGGAGCCAGTAGATCCTAGAC-3') (SEQ ID NO:63)  
 30 EcoRV site underlined, Met-Leu mutation in bold  
 50 nM TAT 3' RV Met-Leu PCR primer  
 (5'-GCTGGATATCCTCTGCTTTGATAGAGAAGC-3') (SEQ ID NO:64)  
 EcoRV site underlined  
 1  $\mu$ L PCR dNTPs



5  $\mu$ L PCR 10X buffer with MgCl<sub>2</sub>

40  $\mu$ L water

1  $\mu$ L Expand polymerase

5 Cycle:

94°C for 5 min

[30 sec 94°C, 1 min 42°C, 1 min 72°C] x 25 cycles

5 min at 72°C

hold at 4°C

10

- The insert was purified over QiaQuick PCR purification column
- The entire reaction was digested with 30 units EcoRV for 3 hours
- The ~200 bp insert was gel purified.
- pcDNA3.1 zeo (+) APP(1-651)/APP(664-695) was digested with EcoRV, and
- 15 then SAP treated
- The Met1-Leu TAT fragment was ligated to pcDNA3.1 zeo (+) APP(1-651)/APP(664-695).

A map of the resulting plasmid is shown in Figure 22C.

20

#### EXAMPLE 7

Design of novel expression vector for expression of APP(1-651)SW, K612V-  
TATexonI(M1L) APP(664-695)

---

- PURPOSE: To provide a low level expression of APP(1-651)SW, K612V-  
TATexonI(M1L) APP(664-695), a prokaryotic selectable marker that is NOT  
25 ampicillin (read-through of the  $\beta$ -lactamase gene is sometimes a problem), and a  
eukaryotic selectable marker that is NOT zeocin (zeo is the marker for the reporter  
plasmids in some embodiments).

#### METHODS

- 30 1. The dEYFP gene was removed from pd2EYFP (Clontech, Palo Alto, CA) using  
BamHI and NotI. The 5' overhangs was filled in using Klenow, and the plasmid was  
re-circularized.

pd2EYFP plasmid was digested with BamHI, NotI.

Ran reaction on 1% agarose gel. Digestion was complete. Cut out 3.4 kb band.

Purified using Qiagen Gel Extraction Kit.

5 Klenow fill-in:

~4 µg plasmid backbone

7.5 µL NEB buffer 2

33 µM each dNTP (diluted from Roche PCR dNTPs)

water to 75 µL

10 4 µL Roche Klenow fragment (4 units)

Incubated at room temperature for 15 minutes

Heat inactivated at 70°C

15 Took 1 µL fill-in reaction.

Diluted to 8 µL with water

Added 2 µL 5X DNA buffer

Added 10 µL 2X Ligation Buffer

Added 1 µL T4 DNA ligase

20

Incubated at room temperature

Transformed 2 µL ligation into Invitrogen maximum efficiency DH5alpha competent cells.

25

Plated out on Kanamycin plates. Lots of colonies.

2. The RSV promoter from pREP4 (Invitrogen) was excised using BglII and HindIII and cloned into the re-circularized plasmid.

30

Digested 5 µg pREP4 with HindIII

Purified using Qiagen PCR purification kit

Digested with BglII.

The RSV promoter fragment was gel purified and cloned into the re-circularized plasmid.

3. The resulting expression vector (pRSV Kan/Neo res; Figure 23) has the eukaryotic RSV promoter 5' to the pd2EYFP polylinker, SV40 driving neo and kanamycin prokaryotic selection, and a pUC ori for high levels of replication in bacteria.

#### EXAMPLE 8

Use of APP(1-651)SW, K612V-TATexonI(M1L) APP(664-695) in HeLa cells with a  $\beta$ -galactosidase reporter gene

The following demonstrates the practice of the present invention with the APP(1-651)SW, K612V-TATexonI(M1L) APP(664-695) fusion protein (SEQ ID NO:2) and  $\beta$ -galactosidase as a reporter gene. P4-R5 cells are HeLa cells that contain a stably integrated  $\beta$ -galactosidase reporter gene under the control of the HIV1 LTR.

#### Materials:

- 1.) Cells: P4-R5 cells
- 2.) DNA: 0.78  $\mu$ g/ $\mu$ L pcDNA3.1 zeo (+)APP(1-651)SW, K612V-TATexonI(M1L) APP(664-695)
- 3.) Transfection reagents: FUGENE®
- 4.) Media: OPTIMEM®  
cDMEM (-)phenol red /10% FBS
- 5.) Compounds: Compound X ( $\beta$ -secretase inhibitor) 10 mM  
L-875,532 ( $\gamma$ -secretase inhibitor) 10 mM

#### Protocol:

##### Day 1

- 1.) Cell count on P4-R5 cells =  $7.6 \times 10^5$  cells per ml in cDMEM (-)PR.  
Seeded sterile white luminometer TC plates with the following cell numbers:

- 10 ml  
 $5 \times 10^3$ /well = 0.75 ml in 9.25 ml media  
 $1.0 \times 10^4$ /well = 1.5 ml in 8.5 ml media

Seeded 100  $\mu$ L cells per well.  
Incubate overnight at 37°C, 5% CO<sub>2</sub>.

### Day 2

2.) Made up media with appropriate dilutions of inhibitors.

5 On no-inhibitor controls, added 100  $\mu$ L of cDMEM with 1% DMSO

On wells with Compound X, added 10  $\mu$ M inhibitor in cDMEM

On wells with L-875,532, added 10  $\mu$ M inhibitor in cDMEM

3.) Prior to transfection, pulled off media on P4-R5 cells and replaced with media +/- inhibitor.

10 FUGENE® transfection:

For FUGENE® transfection:

4.) Added 600  $\mu$ L of OPTIMEM® to sterile EPPENDORF® tube and carefully added 30  $\mu$ L FUGENE® to media, without touching walls of tube. Incubated at room temperature for 5 minutes.

15 In separate EPPENDORF® tubes, added each DNA.

Added FUGENE®/OPTIMEM® dropwise to DNA; incubated at room temperature for 15 minutes.

Added 15  $\mu$ L /well of DNA/FUGENE®/OPTIMEM® dropwise to media in appropriate wells on P4-R5 cells, swirling to mix.

20

TABLE 4

Transfection number	Conc of DNA	Vol of DNA	Vol of FUGENE®	Vol of sterile OPTIMEM®
APP-ML-Tat-APPct	0.78 $\mu$ g/ $\mu$ L	5 $\mu$ g = 6.5 $\mu$ L	30 $\mu$ L of FUGENE®	600 $\mu$ L of OPTIMEM®
pUCd5TAT	1.24 $\mu$ g/ $\mu$ L	5 $\mu$ g = 4.0 $\mu$ L	30 $\mu$ L of FUGENE®	600 $\mu$ L of OPTIMEM®
p243-4	0.56 $\mu$ g/ $\mu$ L	5 $\mu$ g = 9 $\mu$ L	30 $\mu$ L of FUGENE®	600 $\mu$ L of OPTIMEM®

In Table 4, "APP-ML-Tat-APPct" refers to pcDNA3.1 zeo (+)APP(1-651)SW, K612V-TATexonI(M1L) APP(664-695). "pUCd5TAT" is an expression vector that

serves as a positive control for TAT expression, since it is a construct in which TAT is under the control of a strong, constitutive promoter (see Figure 24). "p243-4" is a control expression vector that directs the expression of APP.

- 5 5.) Plates were transferred to an incubator and incubated for 48 hours to allow expression and processing of the proteins.

Day 4

6.) The protocol below was followed for lysis of the cells and measurement of  $\beta$ -galactosidase in the cell lysates.

10

Measurement of  $\beta$ -galactosidase in lysates of transfected cells.

1. Removed TROPIX® chemiluminescence kit(s) from cold room, allowed to come to room temperature in a 37°C water bath.
  2.  $\beta$ -galactosidase standards were prepared:
  - 15 Made 1:5000 dilution of  $\beta$ -galactosidase stock (1 mg/ml) in lysis buffer. Did 2 fold dilutions.
  3. Diluted TROPIX® substrate 1:25 into buffer. (Made enough for 100  $\mu$ L /well).
  4. Added to reservoir and added 100  $\mu$ L/well.
  - 20 5. Added 10  $\mu$ L of  $\beta$ -galactosidase standards to column 12 on plate and incubated in dark for 1 hour.
  6. Read immediately in luminometer using standard file. Filled in required fields, read plate.
- 25 The results are shown in Figure 33. Figure 33 demonstrates that the present invention was able to identify both the  $\beta$ -secretase inhibitor (Compound X) and the  $\gamma$ -secretase inhibitor (L-875,532). In Figure 33, "APP-tat-ct32" refers to pcDNA3.1 zeo (+)APP(1-651)SW, K612V-TATexonI(M1L) APP(664-695). Although not indicated in Figure 33, the results for the controls were as expected: a large transactivation of
- 30 the LTR by pUCd5TAT was observed which was not affected by either inhibitor. No transactivation was seen with p243-4.

## EXAMPLE 9

Comparison of the use of APP(1-651)SW, K612V-TATexonI(M1L) APP(664-695) and APP(1-651)wt, K612V-TATexonI(M1L) APP(664-695) with a  $\beta$ -galactosidase reporter gene

---

- 5                   The following shows a side-by-side comparison of the practice of the present invention with the APP(1-651)SW, K612V-TATexonI(M1L) APP(664-695) fusion protein (SEQ ID NO:2) and the APP(1-651)wt, K612V-TATexonI(M1L) APP(664-695) fusion protein (SEQ ID NO:4). P4-R5 cells are HeLa cells that contain a stably integrated  $\beta$ -galactosidase reporter gene under the control of the HIV1 LTR.

10

Materials:

- 1.) Cells: P4-R5 cells
- 2.) DNA: 0.78  $\mu$ g/ $\mu$ L pcDNA3.1 neo (+) APP(1-651)SW, K612V-TATexonI(M1L) APP(664-695)
- 15                   0.812  $\mu$ g/ $\mu$ L pcDNA3.1 neo (+) APP(1-651)wt, K612V-TATexonI(M1L) APP(664-695)
- 1.24  $\mu$ g/ $\mu$ L pUCd5TAT
- 0.56  $\mu$ g/ $\mu$ L p243-4
- 3.) Transfection reagents: FUGENE®
- 20   4.) Media: OPTIMEM®
- cDMEM (-)phenol red /10% FBS
- 5.) Compounds: Compound X ( $\beta$ -secretase inhibitor) 10 mM
- L-875,532 ( $\gamma$ -secretase inhibitor) 10 mM

Day 1

- 25   1.) Cell count on P4-R5 cells =  $5 \times 10^5$  cells per ml in cDMEM (-)PR.
- Seeded sterile white luminometer TC plates with the following cell numbers:
- 10 ml
- $5 \times 10^3$ /well = 4.0 ml in 36.0 ml media
- Diluted 1:1 into media and seeded one plate at  $2.5 \times 10^3$ /well.
- 30                   Seeded 100  $\mu$ L cells per well.
- Incubated overnight at 37°C, 5% CO<sub>2</sub>.

## Day 2

2.) Made up media with appropriate dilutions of inhibitors.

On no-inhibitor controls, added 100  $\mu$ L of cDMEM with 1% DMSO

On wells with Compound X, added titration curve from 100  $\mu$ M

5 inhibitor in cDMEM (-)PR.

On wells with L-875,532, added titration curve from 100  $\mu$ M inhibitor in cDMEM (-)PR.

3.) Prior to transfection, pulled off media on P4-R5 cells and replaced with media +/-  
10 inhibitor.

FUGENE® transfection:

4.) Added volume of OPTIMEM® to sterile EPPENDORF® tube and carefully  
15 added correct volume of FUGENE® to media, without touching walls of tube.

Incubated at room temperature for 5 minutes.

In separate EPPENDORF® tubes, added each DNA, as outlined below.

Added FUGENE®/OPTIMEM® dropwise to DNA; incubated at room  
temperature for 15 minutes.

20 Added 15  $\mu$ L/well of DNA/FUGENE®/OPTIMEM® dropwise to media in appropriate wells on P4-R5 cells, swirling to mix.

TABLE 5

Transfection number	Conc of DNA	Vol of DNA	Vol of FUGENE®	Vol of sterile OPTIMEM®
1.) APP-ML-Tat-APPct (Sw)	0.78 $\mu$ g/ $\mu$ L	10 $\mu$ g = 13 $\mu$ L	60 $\mu$ L of FUGENE®	1200 $\mu$ L of OPTIMEM®
2.) APP-ML-Tat-APPct (WT)	0.812 $\mu$ g/ $\mu$ L	10 $\mu$ g = 12.2 $\mu$ L	60 $\mu$ L of FUGENE®	1200 $\mu$ L of OPTIMEM®
3.) pUCd5TAT	1.24 $\mu$ g/ $\mu$ L	5 $\mu$ g = 4.0 $\mu$ L	30 $\mu$ L of FUGENE®	600 $\mu$ L of OPTIMEM®
4.) p243-4	0.56 $\mu$ g/ $\mu$ L	5 $\mu$ g = 9 $\mu$ L	30 $\mu$ L of FUGENE®	600 $\mu$ L of OPTIMEM®

In Table 5, "APP-ML-Tat-APPct (Sw)" refers to pcDNA3.1 neo (+) APP(1-651)SW, K612V-TATexonI(M1L) APP(664-695). "APP-ML-Tat-APPct (WT)" refers to pcDNA3.1 neo (+) APP(1-651)wt, K612V-TATexonI(M1L) APP(664-695).

- 5 "pUCd5TAT" is an expression vector that serves as a positive control for TAT expression, since it is a construct in which TAT is under the control of a strong, constitutive promoter (see Figure 24). "p243-4" is a control expression vector that directs the expression of APP.

- 10 5.) Plates were transferred to an incubator and incubated for 36 hours to allow expression and processing of proteins.

#### Day 4

- 15 6.) The protocol below was followed for lysis of the cells and measurement of  $\beta$ -galactosidase in the cell lysates.

#### Measurement of $\beta$ -galactosidase in lysates of transfected cells.

- 20 1. Removed TROPIX® chemiluminescence kit(s) from cold room, allowed to come to room temperature in a 37°C water bath.
2.  $\beta$ -galactosidase standards were prepared:  
Made 1:5000 dilution of  $\beta$ -galactosidase stock (1 mg/ml) in lysis buffer. Did 2 fold dilutions.
- 25 3. Diluted TROPIX® substrate 1:25 into buffer. (Made enough for 120  $\mu$ L /well).
4. Added to reservoir and added 120  $\mu$ L/well.
5. Added 10  $\mu$ L of  $\beta$ -galactosidase standards to column 12 on plate and incubated in dark for 1 hour.
- 30 6. Read immediately in luminometer using standard file. Filled in required fields, read plate.

The results are shown in Figure 34. In Figure 34, "APP(NFEV)HAMycFLAG" refers to a protein that is a variant of APP in which NFEV is present at the  $\beta$ -secretase cleavage site and there are epitope tags in the amino terminal portion of the protein

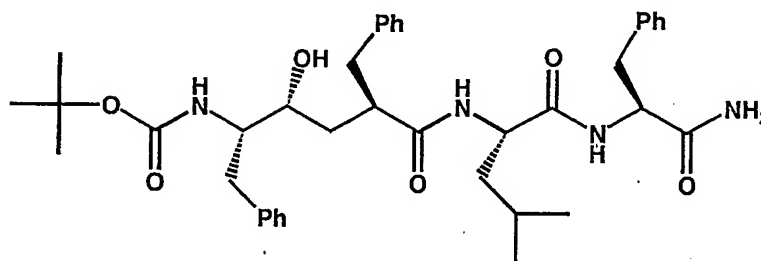


but there is no transcription factor fused to APP. "APP(Sw)tat-ct32" refers to pcDNA3.1 neo (+) APP(1-651)SW, K612V-TATexonI(MIL) APP(664-695). "APP(WT)tat-ct32" refers to pcDNA3.1 neo (+) APP(1-651)wt, K612V-TATexonI(MIL) APP(664-695). Figure 34 shows that the Swedish version and the wild-type version of APP appear to work about equally well in the assay.

## EXAMPLE 10

L-685,458

L-685,458 is a  $\gamma$ -secretase inhibitor having the following structure:



L-685,458 contains an hydroxyethylene dipeptide isostere and is thought to function as a transition state analog mimic of aspartyl proteases (Shearman et al., 2000, Biochemistry 39:8698-8704). L-685,458 was prepared as follows:

{1S-Benzyl-4R-[1-(1S-carbamoyl-2-phenylethylcarbamoyl)-1S-3-methylbutylcarbamoyl]-2R-hydroxy-5-phenylpentyl}carbamic acid tert-butyl ester (L-685,458) was prepared by the coupling of 2R-benzyl-5S-tert-butoxycarbonylamino-4R-(tert-butyltrimethylsilyloxy)-6-phenylhexanoic acid (Evans et al., 1985, J. Org. Chem. 50:4615-4625) with Leu-Phe-NH<sub>2</sub> followed by deprotection with tetrabutylammonium fluoride. The synthesis of {1S-benzyl-4R-[1-(1S-carbamoyl-2-phenylethylcarbamoyl)-1S-3-methylbutylcarbamoyl]-2S-hydroxy-5-phenylpentyl}carbamic acid tert-butyl ester (L-682,679) has been described previously (De Solms et al., 1991, J. Med. Chem. 34:2852-2857). {1S-Benzyl-4R-[1-(1S-carbamoyl-2-phenylethylcarbamoyl)-1S-3-methylbutylcarbamoyl]-2-oxo-5-phenylpentyl}carbamic acid tert-butyl ester (L-684,414) was prepared by pyridinium dichromate-mediated oxidation of L-682,679.

The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from  
5 the foregoing description. Such modifications are intended to fall within the scope of the appended claims.

Various publications are cited herein, the disclosures of which are incorporated by reference in their entireties.

## WHAT IS CLAIMED IS:

1. A DNA molecule comprising a nucleotide sequence encoding a fusion protein comprising amino acids 589-651 selected from the group consisting of wild type APP<sub>695</sub>, the Swedish version of APP<sub>695</sub> and the NFEV (SEQ ID NO:40) version of APP<sub>695</sub> and a transcription factor where the transcription factor is fused in frame to the carboxyl terminus of amino acids 589-651.  
5
2. The DNA molecule of claim 1 where amino acids 589-651 contain a K612V mutation.  
10
3. The DNA molecule of claim 1 where the nucleotide sequence further encodes amino acids 664-695 of APP<sub>695</sub> wherein amino acids 664-695 are fused in frame to the carboxyl terminus of the transcription factor.  
15
4. The DNA molecule of claim 1 where the transcription factor is selected from the group consisting of: HIV-1 TAT, Gal4-VP16, the entire Gal4 protein, LexA-VP16, E12, E47, Twist, Papillomavirus E2, EBV Zta, BIV TAT, HIV-2 TAT, or SIV TAT.  
20
5. The DNA molecule of claim 3 where the fusion protein is selected from the group consisting of: APP(1-651)wt, K612V-TATexonI(M1L) APP (664-695) (SEQ ID NO:4); APP(1-651)wt, K612V, Gal4-VP16(M1L) APP (664-695) (SEQ ID NO:8); APP(1-651)wt, TATexonI(M1L) APP (664-695) (SEQ ID NO:12); and APP(1-651)wt, Gal4-VP16(M1L) APP (664-695) (SEQ ID NO:16).  
25
6. The DNA molecule of claim 3 where the fusion protein is selected from the group consisting of: APP(1-651)SW, K612V-TATexonI(M1L) APP (664-695) (SEQ ID NO:2); APP(1-651)SW, K612V, Gal4-VP16(M1L) APP (664-695) (SEQ ID NO:6); APP(1-651)SW, TATexonI(M1L) APP (664-695) (SEQ ID NO:10); and APP(1-651)SW, Gal4-VP16(M1L) APP (664-695) (SEQ ID NO:14).  
30
7. The DNA molecule of claim 3 where the fusion protein is selected from the group consisting of: APP(1-651)NFEV, K612V-TATexonI(M1L)

APP (664-695) (SEQ ID NO:23) and APP(1-651)NFEV, K612V, GAL4-VP16(M1L)  
APP (664-695) (SEQ ID NO:25).

- 5                   8.     An expression vector comprising the DNA molecule of claim 1.
9.     A eukaryotic cell comprising the DNA molecule of claim 1.
10.    The cell of claim 9 further comprising a reporter gene where the  
10   reporter gene is under the control of a regulatory DNA sequence that is capable of  
being activated by the transcription factor.
11.    A method of identifying a substance that inhibits APP  
processing comprising:
- (a)     providing a recombinant eukaryotic cell which:
- 15                   (i)     expresses a fusion protein comprising amino acids 589-  
651 selected from the group consisting of wild type APP<sub>695</sub>, the Swedish version of  
APP<sub>695</sub> and the NFEV (SEQ ID NO:40) version of APP<sub>695</sub> and a transcription factor  
where the transcription factor is fused in frame to the carboxyl terminus of amino  
acids 589-651; and
- 20                   (ii)    comprises a reporter gene operably linked to a  
regulatory DNA sequence which capable of being activated by the transcription factor;
- (b)     measuring the level of reporter gene product in the cell in the  
absence of the substance;
- (c)     adding the compound to the cell and measuring the level of  
25   reporter gene product in the cell in the presence of the substance;  
where a decrease in the level of reporter gene product in the presence  
as compared to the absence of the substance indicates that the substance inhibits APP  
processing.
- 30                   12.    The method of claim 11 where amino acids 589-651 contain a  
K612V mutation.
13.    The method of claim 11 where the transcription factor is  
selected from the group consisting of: HIV-1 TAT, Gal4-VP16, the entire Gal4

protein, LexA-VP16, E12, E47, Twist, Papillomavirus E2, EBV Zta, BIV TAT, HIV-2 TAT, or SIV TAT.

14. The method of claim 11 where the fusion protein is selected  
5 from the group consisting of: APP(1-651)wt, K612V-TATexonI(M1L) APP (664-695) (SEQ ID NO:4); APP(1-651)wt, K612V, Gal4-VP16(M1L) APP (664-695) (SEQ ID NO:8); APP(1-651)wt, TATexonI(M1L) APP (664-695) (SEQ ID NO:12); and APP(1-651)wt, Gal4-VP16(M1L) APP (664-695) (SEQ ID NO:16).

10 15. The method of claim 11 where the fusion protein is selected from the group consisting of: APP(1-651)SW, K612V-TATexonI(M1L) APP (664-695) (SEQ ID NO:2); APP(1-651)SW, K612V, Gal4-VP16(M1L) APP (664-695) (SEQ ID NO:6); APP(1-651)SW, TATexonI(M1L) APP (664-695) (SEQ ID NO:10); and APP(1-651)SW, Gal4-VP16(M1L) APP (664-695) (SEQ ID NO:14).

15 16. The method of claim 11 where the fusion protein is selected from the group consisting of: APP(1-651)NFEV, K612V-TATexonI(M1L) APP (664-695) (SEQ ID NO:23) and APP(1-651)NFEV, K612V, Gal4-VP16(M1L) APP (664-695) (SEQ ID NO:25).

20 17. A method of identifying a substance that inhibits APP processing comprising:

- (a) providing a recombinant eukaryotic cell which:
  - (i) expresses a fusion protein comprising an amino acid  
25 sequence from APP that is capable of being cleaved by both  $\beta$ -secretase and  $\gamma$ -secretase and a transcription factor where the transcription factor is fused in frame to the amino acid sequence from APP; and
  - (ii) comprises a reporter gene operably linked to a  
regulatory DNA sequence which capable of being activated by the transcription factor;
- 30 (b) measuring the level of reporter gene product in the cell in the absence of the substance;
- (c) adding the compound to the cell and measuring the level of reporter gene product in the cell in the presence of the substance;

where a decrease in the level of reporter gene product in the presence as compared to the absence of the substance indicates that the substance inhibits APP processing.

- 5                    18.    The method of claim 17 where the amino acid sequence from APP comprises an amino acid sequence selected from the group consisting of 589-651 of APP<sub>695</sub>, 589-651 of the Swedish version of APP<sub>695</sub>, and 589-651 of the NFEV version of APP<sub>695</sub>.
- 10                   19.    The method of claim 17 where the amino acid sequence from APP contains the amino acid sequence NLDA (SEQ ID NO:38) at the  $\beta$ -secretase cleavage site instead of the wild-type sequence KMDA (SEQ ID NO:34).
- 15                   20.    The method of claim 17 where the amino acid sequence from APP contains the amino acid sequence NFEV (SEQ ID NO:40) at the  $\beta$ -secretase cleavage site instead of the wild-type sequence KMDA (SEQ ID NO:34).

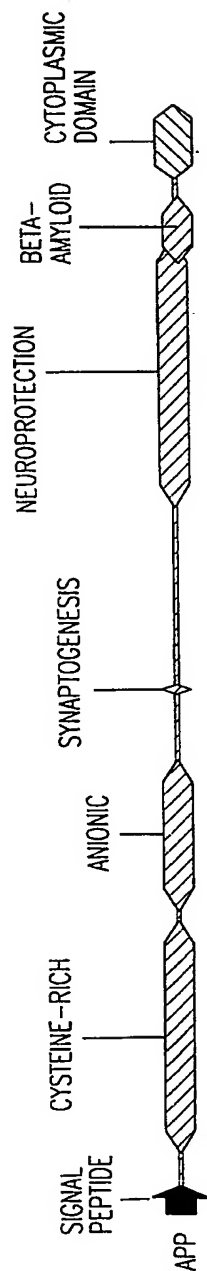


FIG. 1A



FIG. 1B



FIG. 1C



FIG. 1D



FIG. 1E



FIG. 1F



FIG. 1G

2/76

DNA sequence of APP(1-651)SW, K612V-TATexon1(M1 L) APP (664-695)

(SEQ ID NO: 1)

```
1 ATGCTGCCCCG GTTTGGCACT GCTCCTGCTG GCCGCCTGGA CGGCTCGGGC
51 GCTGGAGGTA CCCACTGATG GTAATGCTGG CCTGCTGGCT GAACCCCAAGA
101 TTGCCATGTT CTGTGGCAGA CTGAACATGC ACATGAATGT CCAGAATGGG
151 AAGTGGGATT CAGATCCATC AGGGACCAAA ACCTGCATTG ATACCAAGGA
201 AGGCATCCTG CAGTATTGCC AAGAAGTCTA CCCTGAACTG CAGATCACCA
251 ATGTGGTAGA AGCCAACCAA CCAGTGACCA TCCAGAACTG GTGCAAGCGG
301 GGCCGCAAGC AGTGCAAGAC CCATCCCCAC TTTGTGATTG CCTACCGCTG
351 CTTAGTTGGT GAGTTTATAA GTGATGCCCT TCTCGTTCCT GACAAGTGCA
401 AATTCTTACA CCAGGAGAGG ATGGATGTTT GCGAAACTCA TCTTCACTGG
451 CACACCGTCG CCAAAGAGAC ATGCAGTGAG AAGAGTACCA ACTTGCATGA
501 CTACGGCATG TTGCTGCCCT GCGGAATTGA CAAGTCCGA GGGGTAGAGT
551 TTGTGTGTTG CCCACTGGCT GAAGAAAGTG ACAATGTGGA TTCTGCTGAT
601 GCGGAGGAGG ATGACTCGGA TGTCTGGTGG GCGGAGCAG ACACAGACTA
651 TGCAGATGGG AGTGAAGACA AAGTAGTAGA AGTAGCAGAG GAGGAAGAAG
701 TGGCTGAGGT GGAAGAAGAA GAAGCCGATG ATGACGAGGA CGATGAGGAT
751 GGTGATGAGG TAGAGGAAGA GGCTGAGGAA CCCTACGAAG AAGCCACAGA
801 GAGAACCACC AGCATTGCCA CCACCACCAC CACCACCACA GAGTCTGTGG
851 AAGAGGTGGT TCGAGTTCCT ACAACAGCAG CCAGTACCCC TGATGCCGTT
901 GACAAGTATC TCGAGACACC TGGGGATGAG AATGAACATG CCCATTTCCA
951 GAAAGCCAAA GAGAGGCTTG AGGCCAAGCA CCGAGAGAGA ATGTCCCAGG
1001 TCATGAGAGA ATGGGAAGAG GCAGAACGTC AAGCAAAGAA CTTGCCTAAA
1051 GCTGATAAGA AGGCAGTTAT CCAGCATTTT CAGGAGAAAG TGGAATCTTT
1101 GGAACAGGAA GCAGCCAACG AGAGACAGCA GCTGGTGGAG ACACACATGG
1151 CCAGAGTGGG AGCCATGCTC AATGACCGCC GCCGCCTGGC CCTGGAGAAC
1201 TACATCACCG CTCTGCAGGC TGTTCCTCCT CGGCCTCGTC ACGTGTTCAG
1251 TATGCTAAAG AAGTATGTCC GCGCAGAACA GAAGGACAGA CAGCACACCC
1301 TAAAGCATTT CGAGCATGTG CGCATGGTGG ATCCCAAGAA AGCCGCTCAG
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FIG.2A



3/76

1351 ATCCGGTCCC AGGTTATGAC ACACCTCCGT GTGATTTATG AGCGCATGAA  
1401 TCAGTCTCTC TCCCTGCTCT ACAACGTGCC TGCAGTGGCC GAGGAGATTG  
1451 AGGATGAAGT TGATGAGCTG CTTCAGAAAG AGCAAAACTA TTCAGATGAC  
1501 GTCTTGGCCA ACATGATTAG TGAACCAAGG ATCAGTTACG GAAACGATGC  
1551 TCTCATGCCA TCTTTGACCG AAACGAAAAC CACCGTGGAG CTCCTTCCCG  
1601 TGAATGGAGA GTTCAGCCTG GACGATCTCC AGCCGTGGCA TTCTTTGGG  
1651 GCTGACTCTG TGCCAGCCAA CACAGAAAAC GAAGTTGAGC CTGTTGATGC  
1701 CCGCCCTGCT GCCGACCGAG GACTGACCAC TCGACCAGGT TCTGGGTTGA  
1751 CAAATATCAA GACGGAGGAG ATCTCTGAAG TGAATCTAGA TGCAGAATTC  
1801 CGACATGACT CAGGATATGA AGTTCATCAT CAAGTATTGG TGTTCTTTCG  
1851 AGAAGATGTG GGTTCAAACA AAGGTGCAAT CATTGGACTC ATGGTGGGCG  
1901 GTGTTGTCAT AGCGACAGTG ATCGTCATCA CCTTGGTGAT GCTGAAGAAG  
1951 AAAAAGCTTG GTACCGAGCT CGGATCCACT AGTCCAGTGT GGTGGAATTC  
2001 TGCAGATATC CTGGAGCCAG TAGATCCTAG ACTAGAGCCC TGGAAGCATC  
2051 CAGGAAGTCA GCCTAAAAC GCTTGATCCA ATTGCTATTG TAAAAAGTGT  
2101 TGCTTTCATT GCCAAGTTTG TTTCATGACA AAAGCCTTAG GCATCTCCTA  
2151 TGGCAGGAAG AAGCGGAGAC AGCGACGAAG AGCTCATCAG AACAGTCAGA  
2201 CTCATCAAGC TTCTCTATCA AAGCAGAGGA TATCCAGCAC AGTGGCGGCC  
2251 GCAGACGCCG CTGTCACCCC AGAGGAGCGC CACCTGTCCA AGATGCAGCA  
2301 GAACGGCTAC GAAAATCCAA CCTACAAGTT CTTTGAGCAG ATGCAGAACT  
2351 AG

FIG.2B

4/76

(SEQ ID NO: 2)

Amino acid sequence of APP(1-651)SW, K612V-TATexonI(M1L) APP (664-695)

mlpgla1111aawtaralevptdgnagllaepqiamfcgrlnmhmrvqngkwdsdpstktcidtkegilqycq  
 evypelqitnvveanqpvtiqnwckrgrkqckthphfviyrclvgefisdallvpdkckflhqermdvcethlh  
 whtvaketcsekstnlhdygmllpcgidkfrgvefvccplaeesdnvdsadaeeddsdwwggadtdyadgs  
 edkvvevaeveveveeadddeddgdveveeaeepyeaterettsiatTTTTTtesveevrvpttaastpd  
 avdkyletpgdenehahfqkakerleakhrermsqvmreweeaerqaknlpkadkkaviqhfkvesleqe  
 aanerqqlvethmarveamlndrrrlalenyitalqavpprprhvfmlkkyvraeqkdrqhtlkhfehvmvd  
 pkkaaqrsvmthlrviyermnqslsllnvpavaeeiqdevdelqkeqnysddvlanmiseprisgnda1  
 mpsltetkttvellpvngfslddlqpwhsfgadsvpantenevepvdarpaadrglttrpgsgltnikteeisev  
 2                      3                      4                      5  
 nldaefrhdsgyevhhqylvffaedvgsnkgaiglmvggyviatvivitlvm1kkklgtelgstspwwns  
 6  
 adi1epvdprlepwhpgsqpktactncycckccfhcqvcmfka1qisygrkkrrrrahqnsqthqaslskg  
 7                      8  
 risstvaaadaavtpeerhlskmgqngyenptykffeqmqn

FIG.3

5/76

DNA sequence of APP(1-651)wt, K612V-TATexon1(M1L) APP (664-695)

(SEQ ID NO: 3)

```
1 ATGCTGCCCG GTTTGGCACT GCTCCTGCTG GCCGCCTGGA CGGCTCGGGC
51 GCTGGAGGTA CCCACTGATG GTAATGCTGG CCTGCTGGCT GAACCCAGAG
101 TTGCCATGTT CTGTGGCAGA CTGAACATGC ACATGAATGT CCAGAATGGG
151 AAGTGGGATT CAGATCCATC AGGGACCAAA ACCTGCATTG ATACCAAGGA
201 AGGCATCCTG CAGTATTGCC AAGAAGTCTA CCCTGAACTG CAGATCACCA
251 ATGTGGTAGA AGCCAACCAA CCAGTGACCA TCCAGAACTG GTGCAAGCGG
301 GGCCGCAAGC AGTGCAAGAC CCATCCCCAC TTTGTGATTG CCTACCGCTG
351 CTTAGTTGGT GAGTTTATAA GTGATGCCCT TCTCGTTCCT GACAAGTGCA
401 AATTCTTACA CCAGGAGAGG ATGGATGTTT GCGAACTCA TCTTCACTGG
451 CACACCGTCG CCAAAGAGAC ATGCAGTGAG AAGAGTACCA ACTTGCATGA
501 CTACGGCATG TTGCTGCCCT GCGGAATTGA CAAGTCCGA GGGGTAGAGT
551 TTGTGTGTTG CCCACTGGCT GAAGAAAGTG ACAATGTGGA TTCTGCTGAT
601 GCGGAGGAGG ATGACTCGGA TGTCTGGTGG GCGGAGCAG ACACAGACTA
651 TGCAGATGGG AGTGAAGACA AAGTAGTAGA AGTAGCAGAG GAGGAAGAAG
701 TGGCTGAGGT GGAAGAAGAA GAAGCCGATG ATGACGAGGA CGATGAGGAT
751 GGTGATGAGG TAGAGGAAGA GGCTGAGGAA CCCTACGAAG AAGCCACAGA
801 GAGAACCACC AGCATTGCCA CCACCACCAC CACCACCACA GAGTCTGTGG
851 AAGAGGTGGT TCGAGTTCCT ACAACAGCAG CCAGTACCCC TGATGCCGTT
901 GACAAGTATC TCGAGACACC TGGGGATGAG AATGAACATG CCCATTTCCA
951 GAAAGCCAAA GAGAGGCTTG AGGCCAAGCA CCGAGAGAGA ATGTCCCAGG
1001 TCATGAGAGA ATGGGAAGAG GCAGAACGTC AAGCAAAGAA CTTGCCTAAA
1051 GCTGATAAGA AGGCAGTTAT CCAGCATTTC CAGGAGAAAG TGGAATCTTT
1101 GGAACAGGAA GCAGCCAACG AGAGACAGCA GCTGGTGGAG ACACACATGG
1151 CCAGAGTGGA AGCCATGCTC AATGACCGCC GCCGCCTGGC CCTGGAGAAC
1201 TACATCACCG CTCTGCAGGC TGTTCTCCTT CGGCCTCGTC ACGTGTTCAG
1251 TATGCTAAAG AAGTATGTCC GCGCAGAACA GAAGGACAGA CAGCACACCC
1301 TAAAGCATTT CGAGCATGTG CGCATGGTGG ATCCCAAGAA AGCCGCTCAG
1351 ATCCGGTCCC AGGTTATGAC ACACCTCCGT GTGATTTATG AGCGCATGAA
```

FIG.4A

6/76

1401 TCAGTCTCTC TCCCTGCTCT ACAACGTGCC TGCAGTGGCC GAGGAGATTC  
1451 AGGATGAAGT TGATGAGCTG CTTCAGAAAAG AGCAAACTA TTCAGATGAC  
1501 GTCTTGGCCA ACATGATTAG TGAACCAAGG ATCAGTTACG GAAACGATGC  
1551 TCTCATGCCA TCTTTGACCG AAACGAAAAC CACCGTGGAG CTCCTTCCCG  
1601 TGAATGGAGA GTTCAGCCTG GACGATCTCC AGCCGTGGCA TTCTTTTGGG  
1651 GCTGACTCTG TGCCAGCCAA CACAGAAAAC GAAGTTGAGC CTGTTGATGC  
1701 CCGCCCTGCT GCCGACCGAG GACTGACCAC TCGACCAGGT TCTGGGTTGA  
1751 CAAATATCAA GACGGAGGAG ATCTCTGAAG TGAAGATGGA TGCAGAATTC  
1801 CGACATGACT CAGGATATGA AGTTCATCAT CAAGTATTGG TGTTCTTTGC  
1851 AGAAGATGTG GGTTCAAACA AAGGTGCAAT CATTGGACTC ATGGTGGGCG  
1901 GTGTTGTCAT AGCGACAGTG ATCGTCATCA CCTTGGTGAT GCTGAAGAAG  
1951 AAAAAGCTTG GTACCGAGCT CGGATCCACT AGTCCAGTGT GGTGGAATTC  
2001 TGCAGATATC CTGGAGCCAG TAGATCCTAG ACTAGAGCCC TGGAAGCATC  
2051 CAGGAAGTCA GCCTAAACT GCTTGTACCA ATTGCTATTG TAAAAAGTGT  
2101 TGCTTTCATT GCCAAGTTTG TTTCATGACA AAAGCCTTAG GCATCTCCTA  
2151 TGGCAGGAAG AAGCGGAGAC AGCGACGAAG AGCTCATCAG AACAGTCAGA  
2201 CTCATCAAGC TTCTCTATCA AAGCAGAGGA TATCCAGCAC AGTGGCGGCC  
2251 GCAGACGCCG CTGTCACCCC AGAGGAGCGC CACCTGTCCA AGATGCAGCA  
2301 GAACGGCTAC GAAAATCCAA CCTACAAGTT CTTTGAGCAG ATGCAGAACT  
2351 AG

FIG.4B

FIG. 5

8/76

DNA sequence of APP(1-651)SW, K612V-GAL4VP16(M1 L) APP (664-695)

(SEQ ID NO: 5)

```
1 ATGCTGCCCCG GTTTGGCACT GCTCCTGCTG GCCGCCTGGA CGGCTCGGGC
51 GCTGGAGGTA CCCACTGATG GTAATGCTGG CCTGCTGGCT GAACCCAGA
101 TTGCCATGTT CTGTGGCAGA CTGAACATGC ACATGAATGT CCAGAATGGG
151 AAGTGGGATT CAGATCCATC AGGGACCAAA ACCTGCATTG ATACCAAGGA
201 AGGCATCCTG CAGTATTGCC AAGAAGTCTA CCCTGAACTG CAGATCACCA
251 ATGTGGTAGA AGCCAACCA CCAGTGACCA TCCAGAACTG GTGCAAGCGG
301 GGCCGCAAGC AGTGCAAGAC CCATCCCCAC TTTGTGATTC CCTACCGCTG
351 CTTAGTTGGT GAGTTTATAA GTGATGCCCT TCTCGTTCCT GACAAGTGCA
401 AATTCTTACA CCAGGAGAGG ATGGATGTTT GCGAAACTCA TCTTCACTGG
451 CACACGTCG CCAAAGAGAC ATGCAGTGAG AAGAGTACCA ACTTGCATGA
501 CTACGGCATG TTGCTGCCCT GCGGAATTGA CAAGTCCGA GGGGTAGAGT
551 TTGTGTGTTG CCCACTGGCT GAAGAAAGTG ACAATGTGGA TTCTGCTGAT
601 GCGGAGGAGG ATGACTCGGA TGTCTGGTGG GCGGAGCAG ACACAGACTA
651 TGCAGATGGG AGTGAAGACA AAGTAGTAGA AGTAGCAGAG GAGGAAGAAG
701 TGGCTGAGGT GGAAGAAGAA GAAGCCGATG ATGACGAGGA CGATGAGGAT
751 GGTGATGAGG TAGAGGAAGA GGCTGAGGAA CCCTACGAAG AAGCCACAGA
801 GAGAACCACC AGCATTGCCA CCACCACCAC CACCACCACA GAGTCTGTGG
851 AAGAGGTGGT TCGAGTTCCT ACAACAGCAG CCAGTACCCC TGATGCCGTT
901 GACAAGTATC TCGAGACACC TGGGGATGAG AATGAACATG CCCATTTCCA
951 GAAAGCCAAA GAGAGGCTTG AGGCCAAGCA CCGAGAGAGA ATGTCCCAGG
1001 TCATGAGAGA ATGGGAAGAG GCAGAACGTC AAGCAAAGAA CTTGCCTAAA
1051 GCTGATAAGA AGGCAGTTAT CCAGCATTTT CAGGAGAAAG TGGAATCTTT
1101 GGAACAGGAA GCAGCCAACG AGAGACAGCA GCTGGTGGAG ACACACATGG
1151 CCAGAGTGGA AGCCATGCTC AATGACCGCC GCCGCCTGGC CCTGGAGAAC
1201 TACATCACCG CTCTGCAGGC TGTTCTCCTT CGGCCTCGTC ACGTGTTCAA
1251 TATGCTAAAG AAGTATGTCC GCGCAGAACA GAAGGACAGA CAGCACACCC
```

FIG.6A

9/76

1301 TAAAGCATTT CGAGCATGTG CGCATGGTGG ATCCCAAGAA AGCCGCTCAG  
1351 ATCCGGTCCC AGGTTATGAC ACACCTCCGT GTGATTTATG AGCGCATGAA  
1401 TCAGTCTCTC TCCCTGCTCT ACAACGTGCC TGCAGTGGCC GAGGAGATTC  
1451 AGGATGAAGT TGATGAGCTG CTTCAGAAAG AGCAAACTA TTCAGATGAC  
1501 GTCTTGCCA ACATGATTAG TGAACCAAGG ATCAGTTACG GAAACGATGC  
1551 TCTCATGCCA TCTTTGACCG AAACGAAAAC CACCGTGGAG CTCCTTCCCG  
1601 TGAATGGAGA GTTCAGCCTG GACGATCTCC AGCCGTGGCA TTCTTTTGGG  
1651 GCTGACTCTG TGCCAGCCAA CACAGAAAAC GAAGTTGAGC CTGTTGATGC  
1701 CCGCCCTGCT GCCGACCGAG GACTGACCAC TCGACCAGGT TCTGGGTGA  
1751 CAAATATCAA GACGGAGGAG ATCTCTGAAG TGAATCTAGA TGCAGAATTC  
1801 CGACATGACT CAGGATATGA AGTTCATCAT CAAGTATTGG TGTTCTTTCG  
1851 AGAAGATGTG GGTTCAAACA AAGGTGCAAT CATTGGACTC ATGGTGGGCG  
1901 GTGTTGTCAT AGCGACAGTG ATCGTCATCA CCTTGGTGAT GCTGAAGAAG  
1951 AAAAAGCTTG GTACCGAGCT CGGATCCACT AGTCCAGTGT GGTGGAATTC  
2001 TGCAGATATC AAGCTACTGT CTTCTATCGA ACAAGCATGC GATATTTGCC  
2051 GACTTAAAAA GCTCAAGTGC TCCAAAGAAA AACCGAAGTG CGCCAAGTGT  
2101 CTGAAGAACA ACTGGGAGTG TCGCTACTCT CCCAAAACCA AAAGGTCTCC  
2151 GCTGACTAGG GCACATCTGA CAGAAGTGGA ATCAAGGCTA GAAAGACTGG  
2201 AACAGCTATT TCTACTGATT TTTCTCGAG AAGACCTTGA CATGATTTTG  
2251 AAAATGGATT CTTTACAGGA TATAAAAGCA TTGTTAACAG GATTATTTGT  
2301 ACAAGATAAT GTGAATAAAG ATGCCGTCAC AGATAGATTG GCTTCAGTGG  
2351 AGACTGATAT GCCTCTAACA TTGAGACAGC ATAGAATAAG TGCGACATCA  
2401 TCATCGGAAG AGAGTAGTAA CAAAGGTCAA AGACAGTTGA CTGTATCGGG  
2451 AATTCCCGGG GATCTGGCCC CCCCAGCCGA TGTCAGCCTG GGGGACGAGC  
2501 TCCAATTAGA CGGCGAGGAC GTGGCGATGG CGCATGCCGA CGCGCTAGAC  
2551 GATTTTCGATC TGGACATGTT GGGGGACGGG GATTCCCCGG GTCCGGGATT

FIG.6B

10/76

2601 TACCCCCAC GACTCCGCCC CCTACGGCGC TCTGGATATG GCCGACTTCG  
2651 AGTTTGAGCA GATGTTTACC GATGCCCTTG GAATTGACGA GTACGGTGGG  
2701 GATATCCAGC ACAGTGGCGG CCGCGACGCC GCTGTCACCC CAGAGGAGCG  
2751 CCACCTGTCC AAGATGCAGC AGAACGGCTA CGAAAATCCA ACCTACAAGT  
2801 TCTTTGAGCA GATGCAGAAC TAG

FIG.6C



11/76

(SEQ ID NO: 6)

Amino acid sequence of APP(1-651)SW, K612V, GAL4-VP16(delM1) APP (664-695)

mlpglalllllaawtaralevptdgnagllaepqiamfcgrlnmhmvmqngkwdsdpsgtktcidtkegilqycq  
 evypelqitnvveanqpvtiqnwckrgrkqckthphfviptyrcivgefisdallvpdkckflhqermvdcethlh  
 whtvaketcsekstnlhdygmllpcgidkfrgvefvccplaeedndvsadaeeddsdvwggadtdyadgs  
 edkvvevaeeeeavaeueeeadddeddedgdeveeeaeepyeateerttsiatTTTTTTesveevrvpttaastpd  
 avdkyletpgdenehahfqakerleakhrermsqvmreweeaaerqaknlpkadkkaviqhfhqekvesleqe  
 aanerqqivethmarveamIndrrrlalenyitalqavpprprhvfnnlkkyyvraeqkdrqhtlkhfehvmvd  
 pkkaaqrsvqvmthlrviyermnqslsllnvpavaeeiqdevdelqkeqnysddvlanmisseprisgndal  
 mpsltetktvvelvpngefslddlqpwhsfgadsvpantenevepvdarpaadrglttrpgsgltnikteeisev  
 2 3 4 5  
 nldaefrhdsgyevhhqvlvffaedvgsnkgaiiglmvggvvialviviitlvmllkkklgtelgstspvwns  
adikllssieqacdirciklkskekpkcakclknwecryspktrspitrahltevesrlerleqlfllifpredld  
 6  
milkmDSLQdikaLLtqlfvqdnvkdavtdrlasvetdmpLtlrqhrisatssseessnkqgrqltvsgipqdlapp  
tdvslqdelhldgedvamahadalddfdldmlqgdsgpgqftphdsapygalmdadfeeqmftdalqidev  
 7 8  
ggdiqhsGaaadaavtpeerhlSkmgngyenptykffeqmqn

FIG. 7

12/76

DNA sequence of APP(1-651)wt, K612V, GAL4-VP16(deIM1) APP (664-695)

(SEQ ID NO: 7)

```

1  ATGCTGCCCCG  GTTTGGCACT  GCTCCTGCTG  GCCGCCTGGA  CGGCTCGGGC
51  GCTGGAGGTA  CCCACTGATG  GTAATGCTGG  CCTGCTGGCT  GAACCCAGA
101 TTGCCATGTT  CTGTGGCAGA  CTGAACATGC  ACATGAATGT  CCAGAATGGG
151 AAGTGGGATT  CAGATCCATC  AGGGACCAAA  ACCTGCATTG  ATACCAAGGA
201 AGGCATCCTG  CAGTATTGCC  AAGAAGTCTA  CCCTGAACTG  CAGATCACCA
251 ATGTGGTAGA  AGCCAACCAA  CCAGTGACCA  TCCAGAACTG  GTGCAAGCGG
301 GGCCGCAAGC  AGTGCAAGAC  CCATCCCCAC  TTTGTGATTC  CCTACCGCTG
351 CTTAGTTGGT  GAGTTTATAA  GTGATGCCCT  TCTCGTTCCT  GACAAGTGCA
401 AATTCTTACA  CCAGGAGAGG  ATGGATGTTT  GCGAAACTCA  TCTTCACTGG
451 CACACCGTCG  CCAAAGAGAC  ATGCAGTGAG  AAGAGTACCA  ACTTGCAATG
501 CTACGGCATG  TTGCTGCCCT  GCGGAATTGA  CAAGTTCCGA  GGGGTAGAGT
551 TTGTGTGTTG  CCCACTGGCT  GAAGAAAGTG  ACAATGTGGA  TTCTGCTGAT
601 GCGGAGGAGG  ATGACTCGGA  TGTCTGGTGG  GGCGGAGCAG  ACACAGACTA
651 TGCAGATGGG  AGTGAAGACA  AAGTAGTAGA  AGTAGCAGAG  GAGGAAGAAG
701 TGGCTGAGGT  GGAAGAAGAA  GAAGCCGATG  ATGACGAGGA  CGATGAGGAT
751 GGTGATGAGG  TAGAGGAAGA  GGCTGAGGAA  CCCTACGAAG  AAGCCACAGA
801 GAGAACCACC  AGCATTGCCA  CCACCACCAC  CACCACCACA  GAGTCTGTGG
851 AAGAGGTGGT  TCGAGTTCCT  ACAACAGCAG  CCAGTACCCC  TGATGCCGTT
901 GACAAGTATC  TCGAGACACC  TGGGGATGAG  AATGAACATG  CCCATTTCCA
951 GAAAGCCAAA  GAGAGGCTTG  AGGCCAAGCA  CCGAGAGAGA  ATGTCCCAGG
1001 TCATGAGAGA  ATGGGAAGAG  GCAGAACGTC  AAGCAAAGAA  CTTGCCTAAA
1051 GCTGATAAGA  AGGCAGTTAT  CCAGCATTTT  CAGGAGAAAG  TGAATCTTT
1101 GGAACAGGAA  GCAGCCAACG  AGAGACAGCA  GCTGGTGGAG  ACACACATGG
1151 CCAGAGTGGA  AGCCATGCTC  AATGACCGCC  GCCGCCTGGC  CCTGGAGAAC
1201 TACATACCCG  CTCTGCAGGC  TGTTCCCTCT  CGGCCTCGTC  ACGTGTTCAA

```

FIG.8A

13/76

1251 TATGCTAAAG AAGTATGTCC GCGCAGAACA GAAGGACAGA CAGCACACCC  
1301 TAAAGCATTT CGAGCATGTG CGCATGGTGG ATCCCAAGAA AGCCGCTCAG  
1351 ATCCGGTCCC AGGTTATGAC ACACCTCCGT GTGATTTATG AGCGCATGAA  
1401 TCAGTCTCTC TCCCTGCTCT ACAACGTGCC TGCAGTGGCC GAGGAGATTC  
1451 AGGATGAAGT TGATGAGCTG CTTCAGAAAG AGCAAACTA TTCAGATGAC  
1501 GTCTTGCCA ACATGATTAG TGAACCAAGG ATCAGTTACG GAAACGATGC  
1551 TCTCATGCCA TCTTTGACCG AAACGAAAAC CACCGTGGAG CTCCTTCCCG  
1601 TGAATGGAGA GTTCAGCCTG GACGATCTCC AGCCGTGGCA TTCTTTTGGG  
1651 GCTGACTCTG TGCCAGCCAA CACAGAAAAC GAAGTTGAGC CTGTTGATGC  
1701 CCGCCCTGCT GCCGACCGAG GACTGACCAC TCGACCAGGT TCTGGGTGA  
1751 CAAATATCAA GACGGAGGAG ATCTCTGAAG TGAAGATGGA TGCAGAATTC  
1801 CGACATGACT CAGGATATGA AGTTCATCAT CAAGTATTGG TGTCTTTGC  
1851 AGAAGATGTG GGTTCAAACA AAGGTGCAAT CATTGGACTC ATGGTGGGCG  
1901 GTGTTGTCAT AGCGACAGTG ATCGTCATCA CCTTGGTGAT GCTGAAGAAG  
1951 AAAAAGCTTG GTACCGAGCT CGGATCCACT AGTCCAGTGT GGTGGAATTC  
2001 TGCAGATATC AAGCTACTGT CTTCTATCGA ACAAGCATGC GATATTTGCC  
2051 GACTTAAAAA GCTCAAGTGC TCCAAAGAAA AACC GAAGTG CGCCAAGTGT  
2101 CTGAAGAACA ACTGGGAGTG TCGCTACTCT CCCAAAACCA AAAGGTCTCC  
2151 GCTGACTAGG GCACATCTGA CAGAAGTGGA ATCAAGGCTA GAAAGACTGG  
2201 AACAGCTATT TCTACTGATT TTTCTCGAG AAGACCTTGA CATGATTTTG  
2251 AAAATGGATT CTTTACAGGA TATAAAAGCA TTGTAAACAG GATTATTTGT  
2301 ACAAGATAAT GTGAATAAAG ATGCCGTCAC AGATAGATTG GCTTCAGTGG  
2351 AGACTGATAT GCCTCTAACA TTGAGACAGC ATAGAATAAG TGCGACATCA  
2401 TCATCGGAAG AGAGTAGTAA CAAAGGTCAA AGACAGTTGA CTGTATCGGG  
2451 AATTCCTGGG GATCTGGCCC CCCCACCGA TGTCAGCCTG GGGGACGAGC  
2501 TCCACTTAGA CGGCGAGGAC GTGGCGATGG CGCATGCCGA CGCGCTAGAC  
2551 GATTTGATC TGGACATGTT GGGGGACGGG GATTCCCCGG GTCCGGGATT

FIG.8B

14/76

2601 TACCCCCAC GACTCCGCCC CCTACGGCGC TCTGGATATG GCCGACTTCG  
2651 AGTTTGAGCA GATGTTTACC GATGCCCTTG GAATTGACGA GTACGGTGGG  
2701 GATATCCAGC ACAGTGGCGG CCGCGACGCC GCTGTCACCC CAGAGGAGCG  
2751 CCACCTGTCC AAGATGCAGC AGAACGGCTA CGAAAATCCA ACCTACAAGT  
2801 TCTTTGAGCA GATGCAGAAC TAG

FIG.8C

15/76

(SEQ ID NO: 8)

Amino acid sequence of APP(1-651)wt, K612V, GAL4-VP16(delM1) APP (664-695)

mlpgla1111aawtaralevptdgnagllaepqiamfcgrlnmhmrvqngkwdsdpsgtktcidtkegilqycq  
 evypelqitnvveanqpvtiqnwckrgrkqckthphfviptyrc1vgefisdallvpdkckflhqermdvcethlh  
 whtvaketcsekstnlhdygmllpcgidkfrgvefvccplaeesdnvdsadaeedsdvwggadtdyadgs  
 edkvvevaeeeeaeveeeeeeadddeddedgdeveeeaepeyeaterettsiatTTTTTTesveevrvpttaastpd  
 avdkyletpgdenehahfqkakerleakhrermsqvmreweeaerqaknlpkadkkaviqhfaqekvesleqe  
 aanerqq1vethmarveamlndrrrlalenyitalqavpprprhvfnm1kkyvraeqkdrqht1khfehrmvd  
 pkkaaqrismthlrviyermnqsls1lynpavaeeiqdevde1lkeqnysddv1anmiseprisgndal  
 mps1tetkttve1lpvngefs1dd1qpwhsfgadsvpantenevepvdarpaadrg1ttrpgsg1tnikteeisev  
 2 kmdaefrhdsgeyvhqk1 3 vffaedvgnskgaiig1 4 mvggvviatvivi1 5 tmlkkklgtelgstspwms  
 ad1kl1ssieqacdicr1kk1kcskekpkcakclknwecryspktrsp1trah1tevesr1erleqlf11ifpred1d  
 6 mlkmdslqdikalltqlfvqdnvkdavtdrlasvetdmp1tlrqhrisatssseessnkqgrqltvsqipgd1app  
 tdvslqdelh1dgedvamahadalddfd1dmlqdgdsppgqftphdsapygalmdadfeeqmftdalqidey  
 7 8 ggdiqhsгаааdaavtpeerh1skmqngyenptykffeqmqn

FIG.9

16/76

DNA sequence of APP(1-651)SW, TATexon1(M1 L) APP (664-695)

(SEQ ID NO: 9)

```
1  ATGCTGCCCC  GTTTGGCACT  GCTCCTGCTG  GCCGCCTGGA  CGGCTCGGGC
51  GCTGGAGGTA  CCCACTGATG  GTAATGCTGG  CCTGCTGGCT  GAACCCAGAG
101 TTGCCATGTT  CTGTGGCAGA  CTGAACATGC  ACATGAATGT  CCAGAATGGG
151 AAGTGGGATT  CAGATCCATC  AGGGACCAAA  ACCTGCATTG  ATACCAAGGA
201 AGGCATCCTG  CAGTATTGCC  AAGAAGTCTA  CCCTGAACTG  CAGATCACCA
251 ATGTGGTAGA  AGCCAACCAA  CCAGTGACCA  TCCAGAACTG  GTGCAAGCGG
301 GGCCGCAAGC  AGTGCAAGAC  CCATCCCCAC  TTTGTGATTG  CCTACCGCTG
351 CTTAGTTGGT  GAGTTTATAA  GTGATGCCCT  TCTCGTTCCT  GACAAGTGCA
401 AATTCTTACA  CCAGGAGAGG  ATGGATGTTT  GCGAAACTCA  TCTTCACTGG
451 CACACCGTCG  CCAAAGAGAC  ATGCAGTGAG  AAGAGTACCA  ACTTGCATGA
501 CTACGGCATG  TTGCTGCCCT  GCGGAATTGA  CAAGTCCGA  GGGGTAGAGT
551 TTGTGTGTTG  CCCACTGGCT  GAAGAAAGTG  ACAATGTGGA  TTCTGCTGAT
601 GCGGAGGAGG  ATGACTCGGA  TGTCTGGTGG  GGCGGAGCAG  ACACAGACTA
651 TGCAGATGGG  AGTGAAGACA  AAGTAGTAGA  AGTAGCAGAG  GAGGAAGAAG
701 TGGCTGAGGT  GGAAGAAGAA  GAAGCCGATG  ATGACGAGGA  CGATGAGGAT
751 GGTGATGAGG  TAGAGGAAGA  GGCTGAGGAA  CCCTACGAAG  AAGCCACAGA
801 GAGAACCACC  AGCATTGCCA  CCACCACCAC  CACCACCACA  GAGTCTGTGG
851 AAGAGGTGGT  TCGAGTTCCT  ACAACAGCAG  CCAGTACCCC  TGATGCCGTT
901 GACAAGTATC  TCGAGACACC  TGGGGATGAG  AATGAACATG  CCCATTTCCA
951 GAAAGCCAAA  GAGAGGCTTG  AGGCCAAGCA  CCGAGAGAGA  ATGTCCCAGG
1001 TCATGAGAGA  ATGGGAAGAG  GCAGAACGTC  AAGCAAAGAA  CTTGCCTAAA
1051 GCTGATAAGA  AGGCAGTTAT  CCAGCATTTT  CAGGAGAAAG  TGAATCTTT
1101 GGAACAGGAA  GCAGCCAACG  AGAGACAGCA  GCTGGTGGAG  ACACACATGG
1151 CCAGAGTGGA  AGCCATGCTC  AATGACCGCC  GCCGCCTGGC  CCTGGAGAAC
1201 TACATCACCG  CTCTGCAGGC  TGTTCCTCCT  CGGCCTCGTC  ACGTGTTCAA
```

FIG. 10A

17/76

1251 TATGCTAAAG AAGTATGTCC GCGCAGAACA GAAGGACAGA CAGCACACCC  
1301 TAAAGCATTT CGAGCATGTG CGCATGGTGG ATCCCAAGAA AGCCGCTCAG  
1351 ATCCGGTCCC AGGTTATGAC ACACCTCCGT GTGATTTATG AGCGCATGAA  
1401 TCAGTCTCTC TCCCTGCTCT ACAACGTGCC TGCAGTGGCC GAGGAGATTC  
1451 AGGATGAAGT TGATGAGCTG CTTCAGAAAG AGCAAACTA TTCAGATGAC  
1501 GTCTTGCCA ACATGATTAG TGAACCAAGG ATCAGTTACG GAAACGATGC  
1551 TCTCATGCCA TCTTTGACCG AAACGAAAAC CACCGTGGAG CTCCTTCCCG  
1601 TGAATGGAGA GTTCAGCCTG GACGATCTCC AGCCGTGGCA TTCTTTTGGG  
1651 GCTGACTCTG TGCCAGCCAA CACAGAAAAC GAAGTTGAGC CTGTTGATGC  
1701 CCGCCCTGCT GCCGACCGAG GACTGACCAC TCGACCAGGT TCTGGGTGA  
1751 CAAATATCAA GACGGAGGAG ATCTCTGAAG TGAATCTAGA TGCAGAATTC  
1801 CGACATGACT CAGGATATGA AGTTCATCAT CAAAATTGG TGTCTTTGC  
1851 AGAAGATGTG GGTTCAAACA AAGGTGCAAT CATTGGA CTC ATGGTGGGCG  
1901 GTGTTGTCAT AGCGACAGTG ATCGTCATCA CCTTGGTGAT GCTGAAGAAG  
1951 AAAAAGCTTG GTACCGAGCT CGGATCCACT AGTCCAGTGT GGTGGAATTC  
2001 TGCAGATATC CTGGAGCCAG TAGATCCTAG ACTAGAGCCC TGGGAAGCATC  
2051 CAGGAAGTCA GCCTAAACT GCTTGTACCA ATTGCTATTG TAAAAAGTGT  
2101 TGCTTTCATT GCCAAGTTTG TTTCATGACA AAAGCCTTAG GCATCTCCTA  
2151 TGGCAGGAAG AAGCGGAGAC AGCGACGAAG AGCTCATCAG AACAGTCAGA  
2201 CTCATCAAGC TTCTCTATCA AAGCAGAGGA TATCCAGCAC AGTGGCGGCC  
2251 GCAGACGCCG CTGTCACCCC AGAGGAGCGC CACCTGTCCA AGATGCAGCA  
2301 GAACGGCTAC GAAAATCCAA CCTACAAGTT CTTTGAGCAG ATGCAGAACT  
2351 AG

FIG.10B

18/76

(SEQ ID NO: 10)

Amino acid sequence of APP(1-651)SW, TATexonI(M1L) APP (664-695)

mlpgla1111aawtaralevptdgnagllaepqiamfcgrlnmhmvmqngkwsdpsgtktcidtkegilqycq  
 evypelqitnvveanqpvtiqnwckrgrkqckthphfviptyrc1vgefisdallvpdkckflhqermdvcethlh  
 whtvaketcsekstnlhdygmllpcgidkfrgvefvccplaeesdnvdsadaeeddsdvwggadtdyadgs  
 1  
 edkvvevaeeeeaeveeeeadddeddgdveeeaeepyeeaterttsiatTTTTTTesveevrvpttaastpd  
 avdkyletpgdenehahfqakerleakhrermsqvmreweeaerqaknlpkdkkaviqhfhqekvesleqe  
 aanerqq1vethmarveamlndrrrlalenyitalqavpprprhvfmlkkyvraeqdrqhtlkhfehvrmvd  
 pkkaa1irsqvmthlrviyermnqslsllnvpavaeeiqdevdellqkeqnysddv1anmi sepri sygnda1  
 mpsltetkttvellpvngefslddlqpwhsfgadsvpantenevepvdarpaadrgl1trpgsgltnikteeisev  
 2 3 4 5  
 n1daefrhdsgyevhhqk1lvffaedvgsnkgaiiglmvggv1atv1vitlvm1kkkk1gtelgstspwms  
 6  
 ad1lepvdpr1epwkhpgsqpktactncyckkccfhcqvcmfka1gisyrkrrrrrahqnsqthqas1skq  
 7 8  
 r1sstvaaadaavtpeerhl1skmqngyenptykffeqmqn

FIG. 11



19/76

DNA sequence of APP(1-651)wt, TATexon1(M1L)-APP (664-695)  
(SEQ ID NO: 11)

```
1 ATGCTGCCCC GTTTGGCACT GCTCCTGCTG GCCGCCTGGA CGGCTCGGGC
51 GCTGGAGGTA CCCACTGATG GTAATGCTGG CCTGCTGGCT GAACCCAGAG
101 TTGCCATGTT CTGTGGCAGA CTGAACATGC ACATGAATGT CCAGAATGGG
151 AAGTGGGATT CAGATCCATC AGGGACCAAA ACCTGCATTG ATACCAAGGA
201 AGGCATCCTG CAGTATTGCC AAGAAGTCTA CCCTGAACTG CAGATCACCA
251 ATGTGGTAGA AGCCAACCAA CCAGTGACCA TCCAGAACTG GTGCAAGCGG
301 GGCCGCAAGC AGTGCAAGAC CCATCCCCAC TTTGTGATTG CCTACCGCTG
351 CTTAGTTGGT GAGTTTATAA GTGATGCCCT TCTCGTTCCT GACAAGTGCA
401 AATTCCTACA CCAGGAGAGG ATGGATGTTT GCGAAACTCA TCTTCACTGG
451 CACACGTCG CCAAAGAGAC ATGCAGTGAG AAGAGTACCA ACTTGCATGA
501 CTACGGCATG TTGCTGCCCT GCGGAATTGA CAAGTCCGA GGGGTAGAGT
551 TTGTGTGTTG CCCACTGGCT GAAGAAAGTG ACAATGTGGA TTCTGCTGAT
601 GCGGAGGAGG ATGACTCGGA TGTCTGGTGG GCGGAGCAG ACACAGACTA
651 TGCAGATGGG AGTGAAGACA AAGTAGTAGA AGTAGCAGAG GAGGAAGAAG
701 TGGCTGAGGT GGAAGAAGAA GAAGCCGATG ATGACGAGGA CGATGAGGAT
751 GGTGATGAGG TAGAGGAAGA GGCTGAGGAA CCCTACGAAG AAGCCACAGA
801 GAGAACCACC AGCATTGCCA CCACCACCAC CACCACCACA GAGTCTGTGG
851 AAGAGGTGGT TCGAGTTCCT ACAACAGCAG CCAGTACCCC TGATGCCGTT
901 GACAAGTATC TCGAGACACC TGGGGATGAG AATGAACATG CCCATTTCCA
951 GAAAGCCAAA GAGAGGCTTG AGGCCAAGCA CCGAGAGAGA ATGTCCCAGG
1001 TCATGAGAGA ATGGGAAGAG GCAGAACGTC AAGCAAAGAA CTTGCCTAAA
1051 GCTGATAAGA AGGCAGTTAT CCAGCATTTT CAGGAGAAAG TGGAATCTTT
1101 GGAACAGGAA GCAGCCAACG AGAGACAGCA GCTGGTGGAG ACACACATGG
1151 CCAGAGTGGA AGCCATGCTC AATGACCGCC GCCGCCTGGC CCTGGAGAAC
1201 TACATCACCG CTCTGCAGGC TGTCCTCCT CGGCCTCGTC ACGTGTTCAA
```

FIG.12A

20/76

1251 TATGCTAAAG AAGTATGTCC GCGCAGAACA GAAGGACAGA CAGCACACCC  
1301 TAAAGCATTT CGAGCATGTG CGCATGGTGG ATCCCAAGAA AGCCGCTCAG  
1351 ATCCGGTCCC AGGTTATGAC ACACCTCCGT GTGATTTATG AGCGCATGAA  
1401 TCAGTCTCTC TCCCTGCTCT ACAACGTGCC TGCAGTGGCC GAGGAGATTC  
1451 AGGATGAAGT TGATGAGCTG CTTCAGAAAG AGCAAAACTA TTCAGATGAC  
1501 GTCTTGGCCA ACATGATTAG TGAACCAAGG ATCAGTTACG GAAACGATGC  
1551 TCTCATGCCA TCTTTGACCG AAACGAAAAC CACCGTGGAG CTCCTTCCC  
1601 TGAATGGAGA GTTCAGCCTG GACGATCTCC AGCCGTGGCA TTCTTTTGGG  
1651 GCTGACTCTG TGCCAGCCAA CACAGAAAAC GAAGTTGAGC CTGTTGATGC  
1701 CCGCCCTGCT GCCGACCGAG GACTGACCAC TCGACCAGGT TCTGGGTTGA  
1751 CAAATATCAA GACGGAGGAG ATCTCTGAAG TGAAGATGGA TGCAGAATTC  
1801 CGACATGACT CAGGATATGA AGTTCATCAT CAAAAATTGG TGTTCCTTGC  
1851 AGAAGATGTG GGTTCAAACA AAGGTGCAAT CATTGGACTC ATGGTGGGCG  
1901 GTGTTGTCAT AGCGACAGTG ATCGTCATCA CCTTGGTGAT GCTGAAGAAG  
1951 AAAAAGCTTG GTACCGAGCT CGGATCCACT AGTCCAGTGT GGTGGAATTC  
2001 TGCAGATATC CTGGAGCCAG TAGATCCTAG ACTAGAGCCC TGGAAGCATC  
2051 CAGGAAGTCA GCCTAAACT GCTTGACCA ATTGCTATTG TAAAAAGTGT  
2101 TGCTTTCATT GCCAAGTTTG TTTCATGACA AAAGCCTTAG GCATCTCCTA  
2151 TGGCAGGAAG AAGCGGAGAC AGCGACGAAG AGCTCATCAG AACAGTCAGA  
2201 CTCATCAAGC TTCTCTATCA AAGCAGAGGA TATCCAGCAC AGTGGCGGCC  
2251 GCAGACGCCG CTGTCACCCC AGAGGAGCGC CACCTGTCCA AGATGCAGCA  
2301 GAACGGCTAC GAAAATCCAA CCTACAAGTT CTTTGAGCAG ATGCAGAACT  
2351 AG

FIG.12B

21/76

(SEQ ID NO: 12)

Amino acid sequence of APP(1-651)wt, TATexonI(M1L) APP (664-695)

mlpglailllaawtaralevptdgnagllaepqiamfcgrlnmhmrvqngkwdsdpstktcidtkegilqycq  
 evypelqitnvveanqpvtiqnwckrgrkqckthphfviptyrclvgefisdallvpdkckflhqermdvcethlh  
 whtvaketcsekstnlhdygmllpcgidkfrgvefvccplaeesdnvdsadaeeddsdvwggadtdyadgs  
 edkvvevaeaeveaeveeeadddeddedgdeveeeaeepyeaterttsiatTTTTTTesveevrvpttaastpd  
 avdkyletpgdenehahfqakerleakhrmsqvmreweeaerqaknlpkadkkaviqhfkvesleqe  
 aanerqqlvethmarveamlndrrrlalenyitalqavpprrhvfmlkkyvraeqdrqhtlkhfehvmvd  
 pkkaaqrsvqmthlrviyermnqslsllynvpavaeeiqdevdellqkeqnysddvlanmiseprisgnda  
 mpsltetktvellpvngfslddlqpwhsfgadsvpantenevepvdarpaadrglttrpgsgltnikteeisev  
 2 kmdaefrhdsgeyvhqk 3 lvffaedvgsnkgaiiglmggvv 4 iatvivitlvm 5 lkkklgtelgstspvwns  
 6  
 adilepvdprlepwkhpgsqpktactncycckccfhcqvcmkalqisygrkrrrrahqnsqthqaslskg  
 7 8  
 rissstvaaadaavtpeerhlsmqngnyenptykffeqmqn

FIG. 13

22/76

DNA sequence of APP(1-651)SW, GAL4VP16(delMet) APP (664-695)

(SEQ ID NO: 13)

```
1 ATGCTGCCCC GTTTGGCACT GCTCCTGCTG GCCGCCTGGA CGGCTCGGGC
51 GCTGGAGGTA CCCACTGATG GTAATGCTGG CCTGCTGGCT GAACCCAGA
101 TTGCCATGTT CTGTGGCAGA CTGAACATGC ACATGAATGT CCAGAAATGGG
151 AAGTGGGATT CAGATCCATC AGGGACCAAA ACCTGCATTG ATACCAAGGA
201 AGGCATCCTG CAGTATTGCC AAGAAGCTA CCCTGAACTG CAGATCACCA
251 ATGTGGTAGA AGCCAACCAA CCAGTGACCA TCCAGAACTG GTGCAAGCGG
301 GGCCGCAAGC AGTGCAAGAC CCATCCCCAC TTTGTGATTC CCTACCGCTG
351 CTTAGTTGGT GAGTTTATAA GTGATGCCCT TCTCGTTCCT GACAAGTGCA
401 AATTCTTACA CCAGGAGAGG ATGGATGTTT GCGAAACTCA TCTTCACTGG
451 CACACCGTCG CCAAGAGAGC ATGCAGTGAG AAGAGTACCA ACTTGATGA
501 CTACGGCATG TTGCTGCCCT GCGGAATTGA CAAGTCCGA GGGGTAGAGT
551 TTGTGTGTTG CCCACTGGCT GAAGAAAGTG ACAATGTGGA TTCTGCTGAT
601 GCGGAGGAGG ATGACTCGGA TGTCTGGTGG GGCGGAGCAG ACACAGACTA
651 TGCAGATGGG AGTGAAGACA AAGTAGTAGA AGTAGCAGAG GAGGAAGAAG
701 TGGCTGAGGT GGAAGAAGAA GAAGCCGATG ATGACGAGGA CGATGAGGAT
751 GGTGATGAGG TAGAGGAAGA GGCTGAGGAA CCCTACGAAG AAGCCACAGA
801 GAGAACCACC AGCATTGCCA CCACCACCAC CACCACCACA GAGTCTGTGG
851 AAGAGGTGGT TCGAGTTCCT ACAACAGCAG CCAGTACCCC TGATGCCGTT
901 GACAAGTATC TCGAGACACC TGGGGATGAG AATGAACATG CCCATTTCCA
951 GAAAGCCAAA GAGAGGCTTG AGGCCAAGCA CCGAGAGAGA ATGTCCCAGG
1001 TCATGAGAGA ATGGGAAGAG GCAGAACGTC AAGCAAAGAA CTTGCCTAAA
1051 GCTGATAAGA AGGCAGTTAT CCAGCATTTT CAGGAGAAAG TGGAATCTTT
1101 GGAACAGGAA GCAGCCAACG AGAGACAGCA GCTGGTGGAG ACACACATGG
1151 CCAGAGTGGA AGCCATGCTC AATGACCGCC GCCGCCTGGC CCTGGAGAAC
1201 TACATCACCG CTCTGCAGGC TGTTCTCCTT CGGCCTCGTC ACGTGTTCAA
```

FIG.14A

23/76

1251 TATGCTAAAG AAGTATGTCC GCGCAGAACA GAAGGACAGA CAGCACACCC  
1301 TAAAGCATTT CGAGCATGTG CGCATGGTGG ATCCCAAGAA AGCCGCTCAG  
1351 ATCCGGTCCC AGGTTATGAC ACACCTCCGT GTGATTTATG AGCGCATGAA  
1401 TCAGTCTCTC TCCCTGCTCT ACAACGTGCC TGCAGTGGCC GAGGAGATTC  
1451 AGGATGAAGT TGATGAGCTG CTTCAGAAAG AGCAAACTA TTCAGATGAC  
1501 GTCTTGGCCA ACATGATTAG TGAACCAAGG ATCAGTTACG GAAACGATGC  
1551 TCTCATGCCA TCTTTGACCG AAACGAAAAC CACCGTGGAG CTCCTTCCCG  
1601 TGAATGGAGA GTTCAGCCTG GACGATCTCC AGCCGTGGCA TTCTTTGGG  
1651 GCTGACTCTG TGCCAGCCAA CACAGAAAAC GAAGTTGAGC CTGTTGATGC  
1701 CCGCCCTGCT GCCGACCGAG GACTGACCAC TCGACCAGGT TCTGGGTTGA  
1751 CAAATATCAA GACGGAGGAG ATCTCTGAAG TGAATCTAGA TGCAGAATTC  
1801 CGACATGACT CAGGATATGA AGTTCATCAT CAAAAATTGG TGTCTTTGC  
1851 AGAAGATGTG GGTCAAACA AAGGTGCAAT CATTGGACTC ATGGTGGGCG  
1901 GTGTTGTCAT AGCGACAGTG ATCGTCATCA CCTTGGTGAT GCTGAAGAAG  
1951 AAAAAGCTTG GTACCGAGCT CGGATCCACT AGTCCAGTGT GGTGGAATTC  
2001 TGCAGATATC AAGCTACTGT CTTCTATCGA ACAAGCATGC GATATTTGCC  
2051 GACTTAAAAA GCTCAAGTGC TCCAAAGAAA AACCGAAGTG CGCCAAGTGT  
2101 CTGAAGAACA ACTGGGAGTG TCGCTACTCT CCCAAAACCA AAAGGTCTCC  
2151 GCTGACTAGG GCACATCTGA CAGAAGTGGA ATCAAGGCTA GAAAGACTGG  
2201 AACAGCTATT TCTACTGATT TTTCTCGAG AAGACCTTGA CATGATTTTG  
2251 AAAATGGATT CTTTACAGGA TATAAAAGCA TTGTTAACAG GATTATTTGT  
2301 ACAAGATAAT GTGAATAAAG ATGCCGTAC AGATAGATTG GCTTCAGTGG  
2351 AGACTGATAT GCCTCTAACA TTGAGACAGC ATAGAATAAG TGCGACATCA  
2401 TCATCGGAAG AGAGTAGTAA CAAAGGTCAA AGACAGTTGA CTGTATCGGG  
2451 AATTCCTGGG GATCTGGCCC CCCGACCGA TGTGAGCCTG GGGGACGAGC  
2501 TCCAATTAGA CGGCGAGGAC GTGGCGATGG CGCATGCCGA CGCGCTAGAC  
2551 GATTCGATC TGGACATGTT GGGGACGGG GATTCCCCGG GTCCGGGATT

FIG. 14B

24/76

2601 TACCCCCAC GACTCCGCC CCTACGGCGC TCTGGATATG GCCGACTTCG  
2651 AGTTTGAGCA GATGTTTACC GATGCCCTTG GAATTGACGA GTACGGTGGG  
2701 GATATCCAGC ACAGTGGCGG CCGCGACGCC GCTGTCACCC CAGAGGAGCG  
2751 CCACCTGTCC AAGATGCAGC AGAACGGCTA CGAAAATCCA ACCTACAAGT  
2801 TCTTTGAGCA GATGCAGAAC TAG

FIG.14C

25/76

(SEQ ID NO: 14)

Amino acid sequence of APP(1-651)SW, K612V, GAL4-VP16(delM1) APP (664-695)

mlpgla1111aawtaralevptdgnagllaepqiamfcgrlnmhmrvqngkwdsdpsgtktcidtkegilqycq  
 evypelqitnvveanqpvtiqnwckrgrkqckthphfviptyrc1vgefisdallvpdkckflhqermdvcethlh  
 whtvaketcskstnlhdygmllpcgidkfrgvefvccplaeesdnvdsadaeeddsdvwggadtdyadgs  
 edkvvevaeveveveeadddeddgdveveeeaepeyeaterttsiatTTTTTTesveevrvvpttaastpd  
 avdkyletpgdenehahfqkakerleakhrermsqvmreweeaeqaknlpkdkkaviqhfkqekvesleqe  
 aanerqq1vethmarveamlnrrrrla1enyitalqavpprprhvfmm1kkyvraeqkdrqht1khfehvmvd  
 pkkaaqrsvmthlrviyermnqsls1lynvpavaeeiqdevdellqkeqnysddv1anmiseprisgnda1  
 mps1tetkttvellpvngfsl1dd1qpwhsfgadsvpantenevepydarpaadrglttrpgsgltnikteeisev  
 2 n1daefrhdsgeyevhhqk1vffaedvgsnkgaiiglmvggvv1atvivit1vm1kkkk1gtelgstspwms  
 3 4 5  
 ad+k11ss1eqacdicr1kk1kcskekpkcakclknwecryspktrsp1trah1tevesr1er1eq1f111fpred1d  
 6  
 milkm1slqdikalltqlfvqdnvknkdavtdr1asvetdmp1tlrqhrisatssseessnkqqrqltvsgipqdlapp  
 7 8  
 tdvslgdelh1dgedvamahada1ddfd1dm1gdgdsppgftphdsapygalmdadfeeqmftdalqidey  
 ggdiqhs1gaaadaavtpeerh1skmqngyenptykffeqmqn

FIG. 15

26/76

DNA sequence of APP(1-651)wt, GAL4VP16(delMet) APP (664-695)

(SEQ ID NO: 15)

```
1 ATGCTGCCCC GTTTGGCACT GCTCCTGCTG GCCGCCTGGA CGGCTCGGGC
51 GCTGGAGGTA CCCACTGATG GTAATGCTGG CCTGCTGGCT GAACCCAGAG
101 TTGCCATGTT CTGTGGCAGA CTGAACATGC ACATGAATGT CCAGAATGGG
151 AAGTGGGATT CAGATCCATC AGGGACCAAA ACCTGCATTG ATACCAAGGA
201 AGGCATCCTG CAGTATTGCC AAGAAGCTA CCCTGAACTG CAGATCACCA
251 ATGTGGTAGA AGCCAACCAA CCAGTGACCA TCCAGAACTG GTGCAAGCGG
301 GGCCGCAAGC AGTGCAAGAC CCATCCCCAC TTTGTGATTG CCTACCGCTG
351 CTTAGTTGGT GAGTTTATAA GTGATGCCCT TCTCGTTCCT GACAAGTGCA
401 AATTCTTACA CCAGGAGAGG ATGGATGTTT GCGAAACTCA TCTTCACTGG
451 CACACCGTCG CCAAAGAGAC ATGCAGTGAG AAGAGTACCA ACTTGCAATG
501 CTACGGCATG TTGCTGCCCT GCGGAATTGA CAAGTTCCGA GGGGTAGAGT
551 TTGTGTGTTG CCCACTGGCT GAAGAAAGTG ACAATGTGGA TTCTGCTGAT
601 GCGGAGGAGG ATGACTCGGA TGTCTGGTGG GGCGGAGCAG ACACAGACTA
651 TGCAGATGGG AGTGAAGACA AAGTAGTAGA AGTAGCAGAG GAGGAAGAAG
701 TGGCTGAGGT GGAAGAAGAA GAAGCCGATG ATGACGAGGA CGATGAGGAT
751 GGTGATGAGG TAGAGGAAGA GGCTGAGGAA CCCTACGAAG AAGCCACAGA
801 GAGAACCACC AGCATTGCCA CCACCACCAC CACCACCACA GAGTCTGTGG
851 AAGAGGTGGT TCGAGTTCCT ACAACAGCAG CCAGTACCCC TGATGCCGTT
901 GACAAGTATC TCGAGACACC TGGGGATGAG AATGAACATG CCCATTTCCA
951 GAAAGCCAAA GAGAGGCTTG AGGCCAAGCA CCGAGAGAGA ATGTCCCAGG
1001 TCATGAGAGA ATGGGAAGAG GCAGAACGTC AAGCAAAGAA CTTGCCTAAA
1051 GCTGATAAGA AGGCAGTTAT CCAGCATTTT CAGGAGAAAG TGGAATCTTT
1101 GGAACAGGAA GCAGCCAACG AGAGACAGCA GCTGGTGGAG ACACACATGG
1151 CCAGAGTGGA AGCCATGCTC AATGACCGCC GCCGCCTGGC CCTGGAGAAC
1201 TACATCACCG CTCTGCAGGC TGTTCTCCTCT CGGCCTCGTC ACGTGTTCAA
```

FIG. 16A



27/76

1251 TATGCTAAAG AAGTATGTCC GCGCAGAACA GAAGGACAGA CAGCACACCC  
1301 TAAAGCATTT CGAGCATGTG CGCATGGTGG ATCCCAAGAA AGCCGCTCAG  
1351 ATCCGGTCCC AGGTTATGAC ACACCTCCGT GTGATTTATG AGCGCATGAA  
1401 TCAGTCTCTC TCCCTGCTCT ACAACGTGCC TGCAGTGGCC GAGGAGATTC  
1451 AGGATGAAGT TGATGAGCTG CTTCAGAAAG AGCAAAACTA TTCAGATGAC  
1501 GTCTTGGCCA ACATGATTAG TGAACCAAGG ATCAGTTACG GAAACGATGC  
1551 TCTCATGCCA TCTTTGACCG AAACGAAAAC CACCGTGGAG CTCCTTCCCG  
1601 TGAATGGAGA GTTCAGCCTG GACGATCTCC AGCCGTGGCA TTCTTTTGGG  
1651 GCTGACTCTG TGCCAGCCAA CACAGAAAAC GAAGTTGAGC CTGTTGATGC  
1701 CCGCCCTGCT GCCGACCGAG GACTGACCAC TCGACCAGGT TCTGGGTTGA  
1751 CAAATATCAA GACGGAGGAG ATCTCTGAAG TGAAGATGGA TGCAGAATTC  
1801 CGACATGACT CAGGATATGA AGTTCATCAT CAAAAATTGG TGTTCTTTGC  
1851 AGAAGATGTG GGTTCAAACA AAGGTGCAAT CATTGGACTC ATGGTGGGCG  
1901 GTGTTGTCAT AGCGACAGTG ATCGTCATCA CCTTGGTGAT GCTGAAGAAG  
1951 AAAAAGCTTG GTACCGAGCT CGGATCCACT AGTCCAGTGT GGTGGAATTC  
2001 TGCAGATATC AAGCTACTGT CTTCTATCGA ACAAGCATGC GATATTTGCC  
2051 GACTTAAAAA GCTCAAGTGC TCCAAAGAAA AACC GAAGTG CGCCAAGTGT  
2101 CTGAAGAACA ACTGGGAGTG TCGTACTCT CCCAAAACCA AAAGGTCTCC  
2151 GCTGACTAGG GCACATCTGA CAGAAGTGGA ATCAAGGCTA GAAAGACTGG  
2201 AACAGCTATT TCTACTGATT TTTCCTCGAG AAGACCTTGA CATGATTTTG  
2251 AAAATGGATT CTTTACAGGA TATAAAAGCA TTGTTAACAG GATTATTTGT  
2301 ACAAGATAAT GTGAATAAAG ATGCCGTCAC AGATAGATTG GCTTCAGTGG  
2351 AGACTGATAT GCCTCTAACA TTGAGACAGC ATAGAATAAG TGCGACATCA  
2401 TCATCGGAAG AGAGTAGTAA CAAAGGTCAA AGACAGTTGA CTGTATCGGG  
2451 AATTCCCGGG GATCTGGCCC CCCC GACCGA TGTCAGCCTG GGGGACGAGC  
2501 TCCACTTAGA CGGCGAGGAC GTGGCGATGG CGCATGCCGA CGCGCTAGAC  
2551 GATTTTCGATC TGGACATGTT GGGGGACGGG GATTCCCCGG GTCCGGGATT

FIG.16B

28/76

2601 TACCCCCAC GACTCCGCCC CCTACGGCGC TCTGGATATG GCCGACTTCG  
2651 AGTTTGAGCA GATGTTTACC GATGCCCTTG GAATTGACGA GTACGGTGGG  
2701 GATATCCAGC ACAGTGGCGG CCGCGACGCC GCTGTCACCC CAGAGGAGCG  
2751 CCACCTGTCC AAGATGCAGC AGAACGGCTA CGAAAATCCA ACCTACAAGT  
2801 TCTTTGAGCA GATGCAGAAC TAG

FIG.16C

29/76

(SEQ ID NO: 16)

Amino acid sequence of APP(1-651)wt, K612V, GAL4-VP16(delM1) APP (664-695)

mlpgla1111aawtaralevptdgnagllaepqiamfcgrlnmhmrvqngkwdsdpsgtktcidtkegilqycq  
 evypelqitnvveanqpvtiqnwckrgrkqckthphfviyrc1vgefisdallvpdkckflhqermdvcethlh  
 whtvaketcskstnlhdygmllpcgidkfrgvefvccplaesdnvdsadaeedsdvwggadtdyadgs  
 edkvvevaeeeeaeveeeeeadddedgedeveeeaepeyeaterettsiatTTTTTTesveevrvpttaastpd  
 avdkyletpgdenehahfqkakerleakhrermsqvmreweeaerqaknlpkadkkaviqhfkvesleqe  
 aanerqq1vethmarveamlnrrrrla1enyitalqavpprprhvfmlkkyvraeqkdrqhtlkhfehrvmvd  
 pkkaaqrismthlrviyermnqsls1lynvpaeeiqdevdellqkeqnysddv1anmiseprisgnda1  
 mpsltetktte1lpvngefs1dd1qpwhsfgadsvpantenevepvdarpaadrglttrpgsgltnikteeisev  
 2 kmdaefrhdsgeyvhqklvffaedvgsnkgaiiglmvggvviatvivitlvmkkkk1gtele1gstspwms  
 adik11ssieqacd1ck1k1kskekpkcakclknwecryspktrsp1trahltevesr1erleqlf11ifpred1d  
 6 milkmdslqdika11tqlfvqdnvkdavtdrlasvetdmp1tlrqhrisatssseessnkqqrqltvsgipqdlapp  
 tdvslqdelh1dgedvamahada1ddfd1dm1gdqdsppgpgftphdsapygalmdadfeeqmftdalqidey  
 7 8 ggdiqhsaaaadaavtpeerh1skmqngyenptykffeqmqn

FIG.17

30/76

(SEQ ID NO: 17)

```
1 agtttcctcg gcagcggtag gcgagagcac gcggaggagc gtgcgcgggg gccccgggag
61 acggcgccgg tggcgccgag ggcagagcaa ggacgcggcg gatcccactc gcacagcagc
121 gcactcgggtg ccccgccgag ggtcgcgatg ctgcccgggt tggcactgct cctgctggcc
181 gcctggacgg ctcgggcgct ggaggtaccc actgatggta atgctggcct gctggctgaa
241 cccagattg ccatgttctg tggcagactg aacatgcaca tgaatgtcca gaatgggaag
301 tgggattcag atccatcagg gacaaaacc tgcattgata ccaaggaagg catcctgcag
361 tattgccaag aagtctaccc tgaactgcag atcaccaatg tggtagaagc caaccaacca
421 gtgaccatcc agaactgggt caagcggggc cgcaagcagt gcaagacca tccccacttt
481 gtgattccct accgctgctt agttggtgag tttgtaagtg atgcccttct cgttcctgac
541 aagtgcaa atcttacacca ggagaggatg gatgtttgcg aaactcatct tcaactggcac
601 accgtcgcca aagagacatg cagtgaaga agtaccactg tgcattgacta cggcatgttg
661 ctgccctgcg gaattgacaa gttccgaggg gtagagtttg tgtgttgccc actggctgaa
721 gaaagtgaca atgtggattc tgctgatgcg gaggaggatg actcggatgt ctggtggggc
781 ggagcagaca cagactatgc agatgggagt gaagacaaag tagtagaagt agcagaggag
841 gaagaagtgg ctgagtgga agaagaaga gccgatgatg acgaggacga tgaggatggt
901 gatgaggtag aggaagaggc tgaggaaccc tacgaagaag ccacagagag aaccaccagc
961 attgccacca ccaccaccac caccacagag tctgtggaag aggtggttcg agttcctaca
1021 acagcagcca gtaccctga tgccgttgac aagtatctcg agacacctgg ggatgagaat
1081 gaacatgccc atttccagaa agccaaagag aggttgagg ccaagcaccg agagagaatg
1141 tcccaggtca tgagagaatg ggaagaggca gaacgtcaag caaagaactt gcciaaagct
1201 gataagaagg cagttatcca gcatttccag gagaaagtgg aatctttgga acaggaagca
1261 gccaacgaga gacagcagct ggtggagaca cacatggcca gagtggaagc catgctcaat
1321 gaccgcccgc gcctggccct ggagaactac atcacgctc tgcaggctgt tcctcctcgg
1381 cctcgtaacg tgttcaatat gctaaagaag tatgtccgag cagaacagaa ggacagacag
1441 cacaccctaa agcatttcga gcatgtgcgc atggtggatc ccaagaaagc cgctcagatc
1501 cgggtcccag ttatgacaca cctccgtgtg atttatgagc gcatgaatca gtctctctcc
1561 ctgctctaca acgtgcctgc agtgccgag gagattcagg atgaagtga tgagctgctt
1621 cagaaagagc aaaactattc agatgacgtc ttggccaaca tgattagtga accaaggatc
1681 agttacggaa acgatgctct catgccatct ttgaccgaaa cgaaaaccac cgtggagctc
1741 cttcccgtga atggagagtt cagcctggac gatctccagc cgtggcattc ttttggggct
1801 gactctgtgc cagccaacac agaaaacgaa gttgagcctg ttgatgcccg ccctgctgcc
1861 gaccgaggac tgaccactcg accaggttct gggttgacaa atatcaagac ggaggagatc
1921 tctgaagtga agatggatgc agaattccga catgactcag gatatgaagt tcatcatcaa
1981 aaattggtgt tctttgcaga agatgtgggt tcaaacaaag gtgcaatcat tggactcatg
2041 gtgggcgggt ttgtcatagc gacagtgatc gtcacacact tgggtgatgt gaagaagaaa
2101 cagtacacat ccattcatca tgggtgtgtg gaggttgacg ccgctgtcac cccagaggag
2161 cgccacctgt ccaagatgca gcagaacggc tacgaaaatc caacctacaa gttctttgag
2221 cagatgcaga actagacccc cgccacagca gcctctgaag ttggacagca aaaccattgc
2281 ttcactaccc atcggtgtcc atttatagaa taatgtggga agaaacaaac ccgttttatg
2341 atttactcat tatcgctttt tgacagctgt gctgtaacac aagtagatgc ctgaacttga
2401 attaatccac acatcagtaa tgtattctat ctctctttac attttggctc ctatactaca
2461 ttattaatgg gttttgtgta ctgtaaagaa tttagctgta tcaaactagt gcatgaatag
2521 attctctcct gattatttat cacatagccc cttagccagt tgtatattat tcttgtggtt
2581 tgtgacccaa ttaagtccta ctttacatat gctttaagaa tcgatggggg atgcttcag
2641 tgaacgtggg agttcagctg cttctcttgc ctaagtattc ctttctgat cactatgcat
2701 tttaaagtta aacattttta agtatttcag atgctttaga gagatttttt ttccatgact
2761 gcattttact gtacagattg ctgcttctgc tatatttgtg atataggaat taagaggata
```

FIG. 18A

31/76

2821 cacacgtttg tttcttcgtg cctgttttat gtgcacacat taggcattga gacttcaagc  
2881 ttttcttttt ttgtccacgt atctttgggt ctttgataaa gaaaagaatc cctgttcatt  
2941 gtaagcactt ttacggggcg ggtggggagg ggtgctctgc tggcttcaa ttaccaagaa  
3001 ttctccaaaa caattttctg caggatgatt gtacagaatc attgcttatg acatgatcgc  
3061 tttctacact gtattacata aataaattaa ataaaataac cccgggcaag acttttcttt  
3121 gaaggatgac tacagacatt aaataatcga agtaattttg ggtggggaga agaggcagat  
3181 tcaattttct ttaaccagtc tgaagtttca tttatgatac aaaagaagat gaaaatggaa  
3241 gtggcaatat aaggggatga ggaaggcatg cctggacaaa cccttctttt aagatgtgtc  
3301 ttcaatttgt ataaaatggt gttttcatgt aaataaatac attcttgag gagc

FIG. 18B

32/76

(SEQ ID NO: 18)

MLPGLALLLLAAWTARALEVPTDGNAGLLAEPQIAMFCGRLNMH  
MNVQNGKWDSDPSGKTCIDTKEGILQYCQEVPELQITNVVEANQPVTIQNWCKRGR  
KQCKTHPHFVIPYRCLVGEFVSDALLVPDKCKFLHQERMDVCETHLHWHTVAKETCSE  
KSTNLHDYGMLLPCGIDKFRGVFVCCPLAEESDNVDSADAEEDDSVWVGADTDYA  
DGSEDKVVEVAEEEEVAVEEEEEADDEDEDGDEVEEEAEPEYEEATERTTSIATTT  
TTTTESVEEVVRVPTTAASTPDAVDKYLETPGDENEHAHFQKAKERLEAKHRERMSQV  
MREWEAERQAKNLPKADKKAVIQHFQEKVESLEQEAAERQQLVETHMARVEAMLND  
RRRLALENYITALQAVPPRPRHVFNMLKKYVRAEQKDRQHTLKHFEHVRMVDPKKAQ  
IRSQVMTHLRVIYERMNQSLSLLYNVPAVAEEIQDEVDELLQKEQNYSDVLANMISE  
PRISYGNDAIMPSTETKTTVELLPVNGEFSDDLQPWHSFGADSVANTENEVEPVD  
ARPAADRGLTTRPGSGLTNIKTEEISEVKMDAÉFRHDSGYEVHHQKLVFFAEDVGSNK  
GAIIGLMVGGVVIATVIVITLVMLKKKQYTSIHGVEVDAAVTPEERHLSKMQQNGY  
ENPTYKFFEQMQN

FIG. 18C

33/76

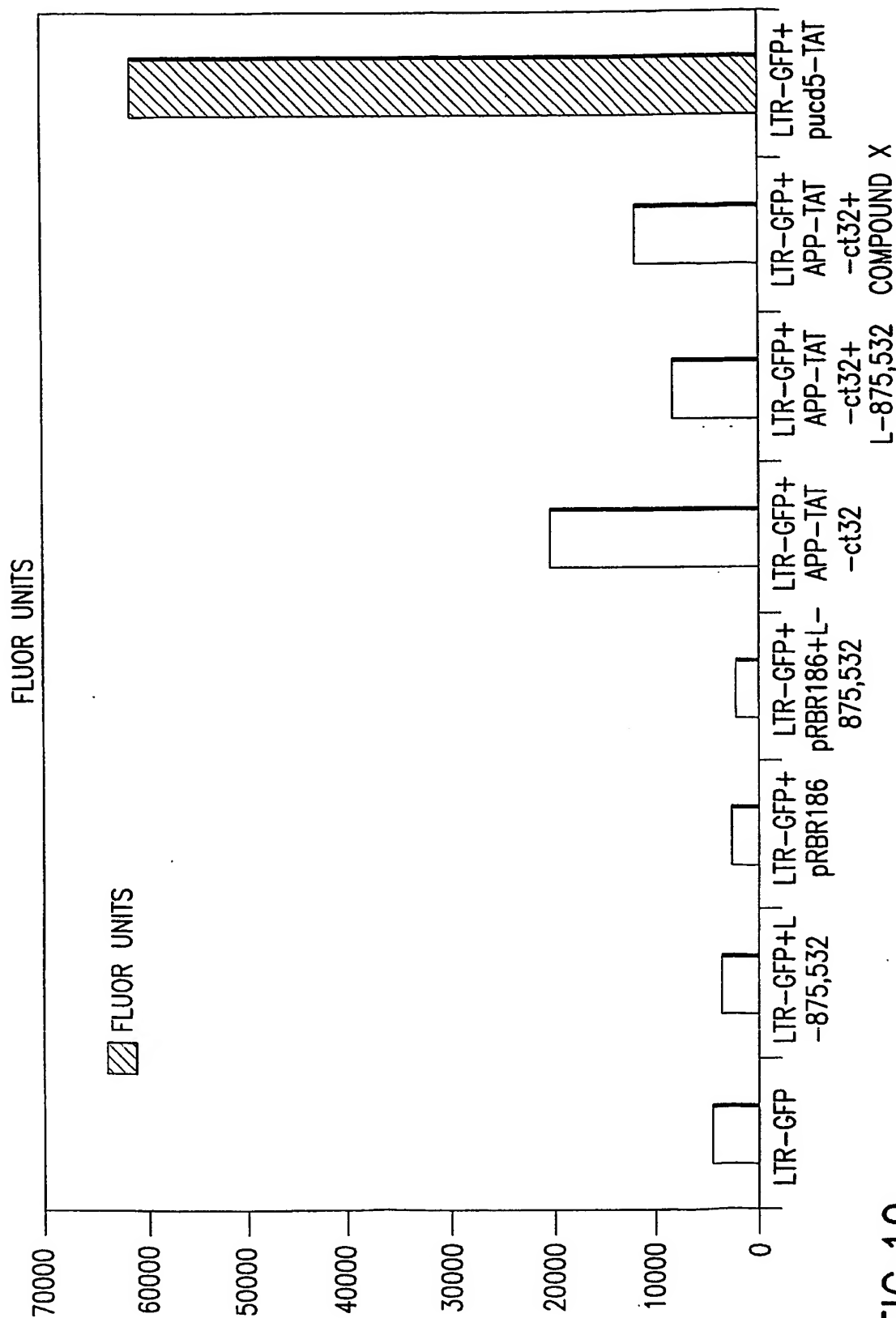


FIG.19

34/76

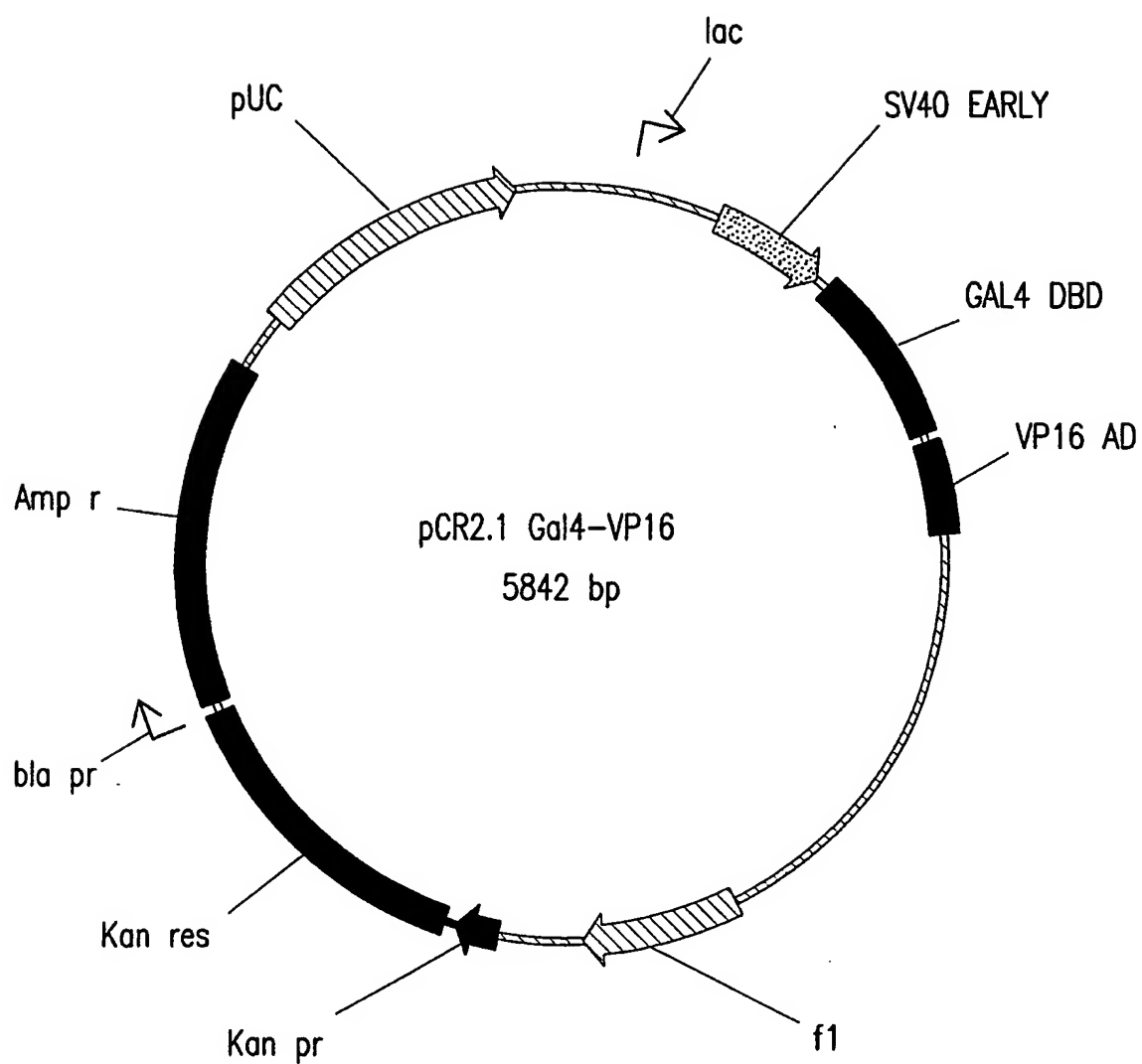


FIG.20



35/76

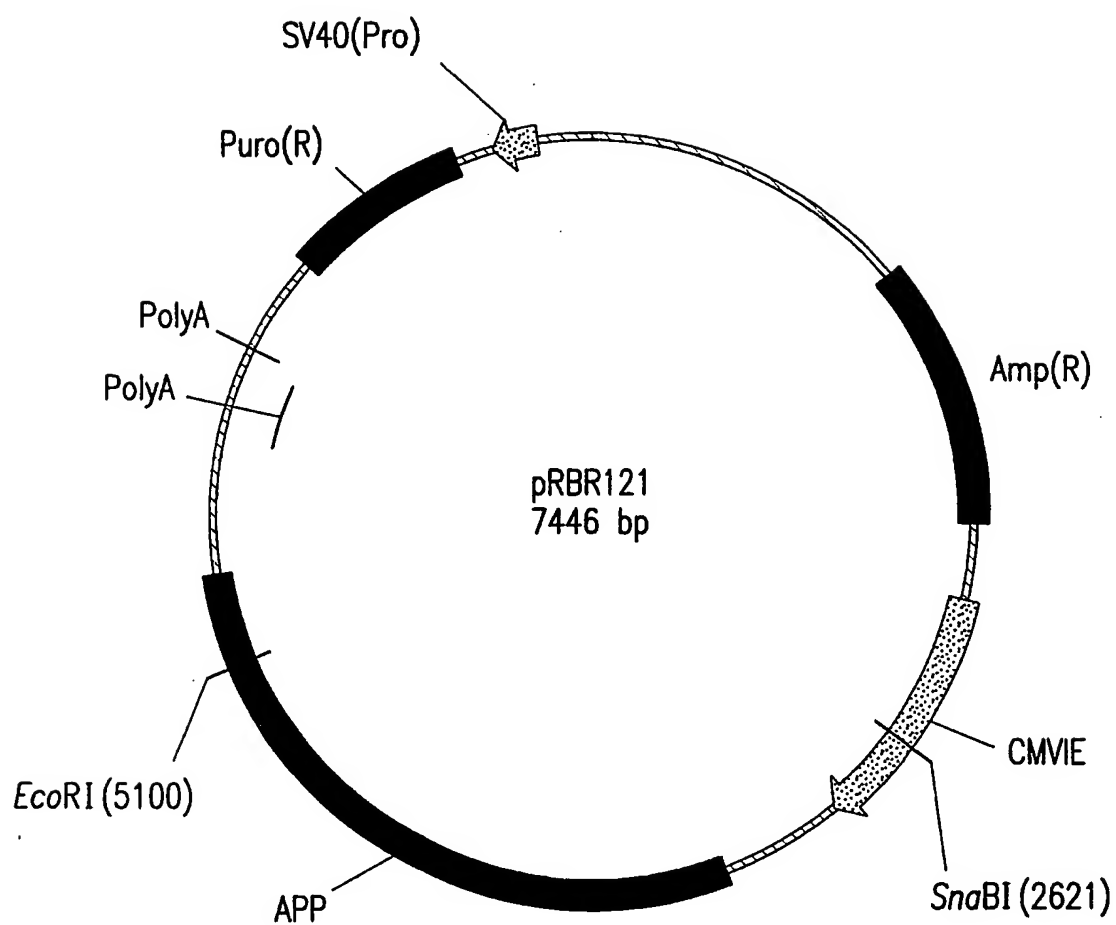


FIG.21A

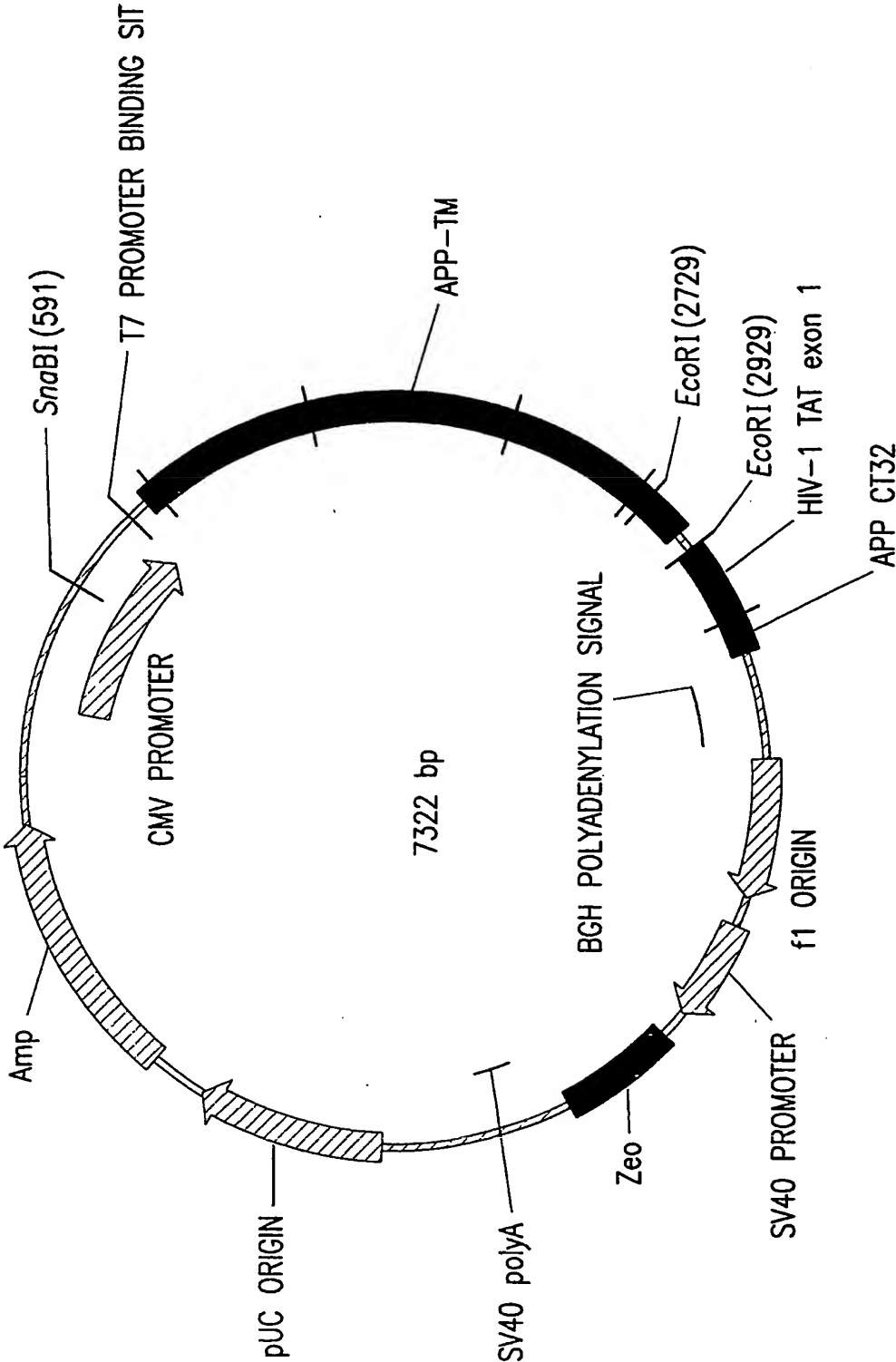


FIG.21B

37/76

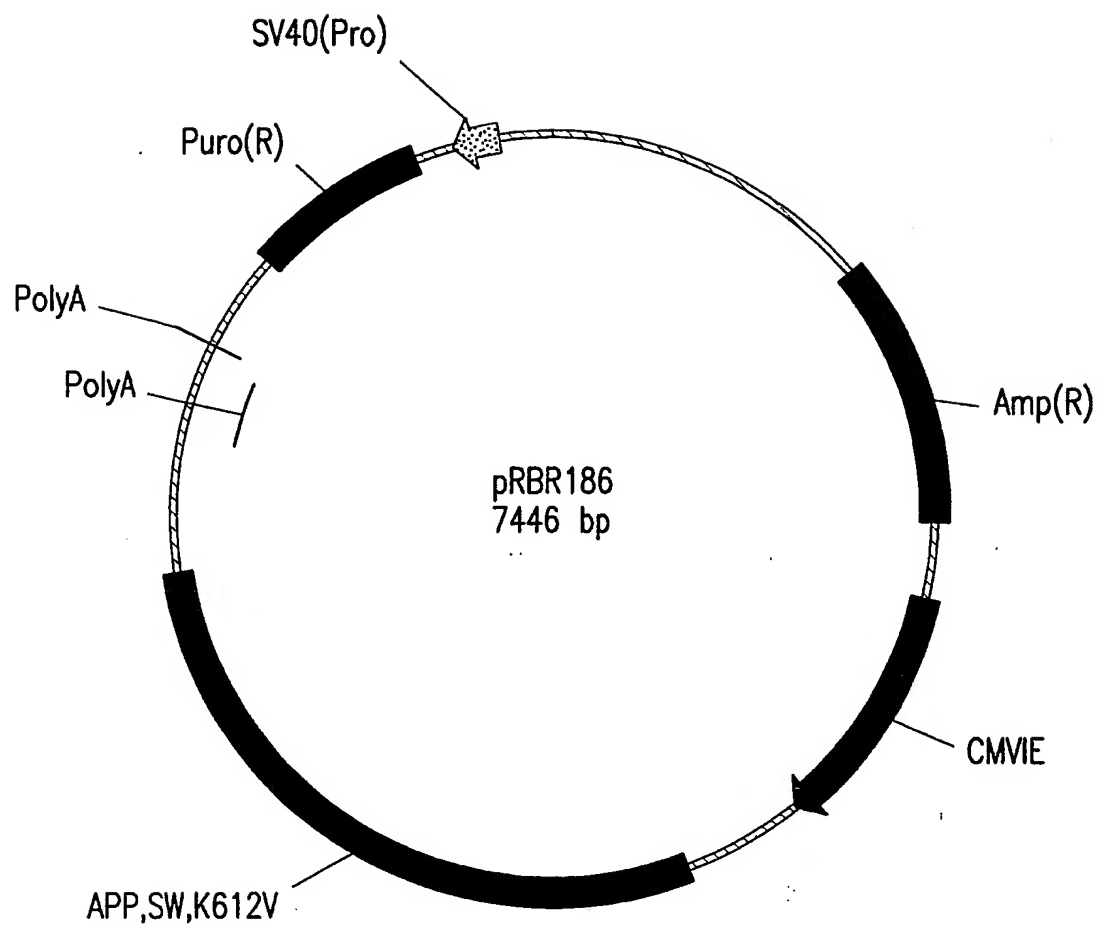
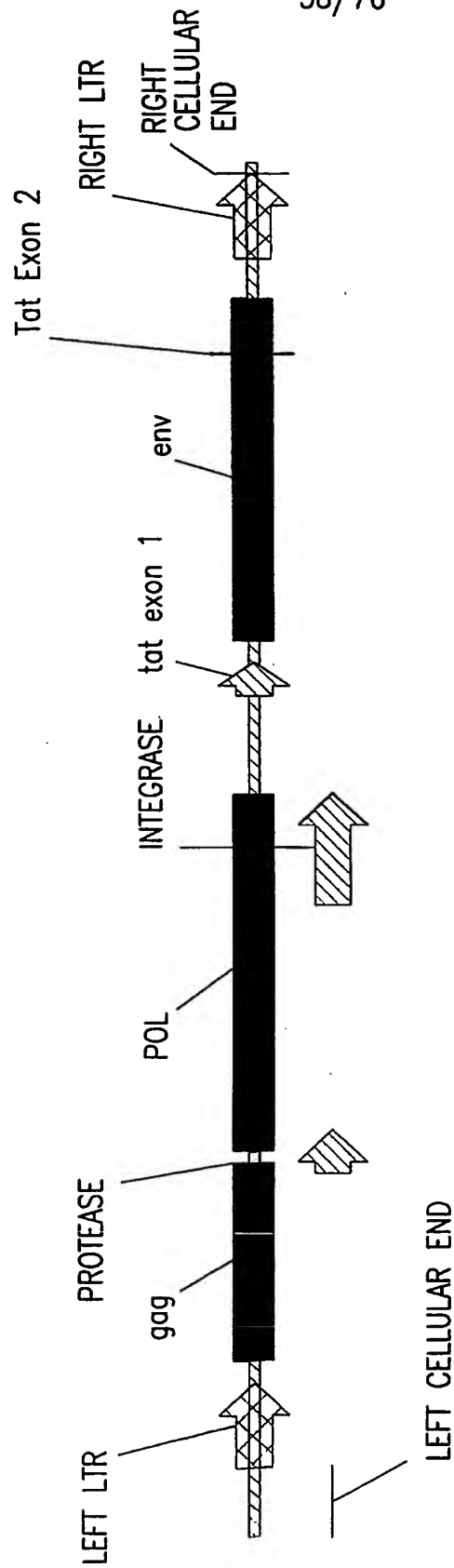


FIG.22A

38/76



10317 bp

FIG.22B

39/76

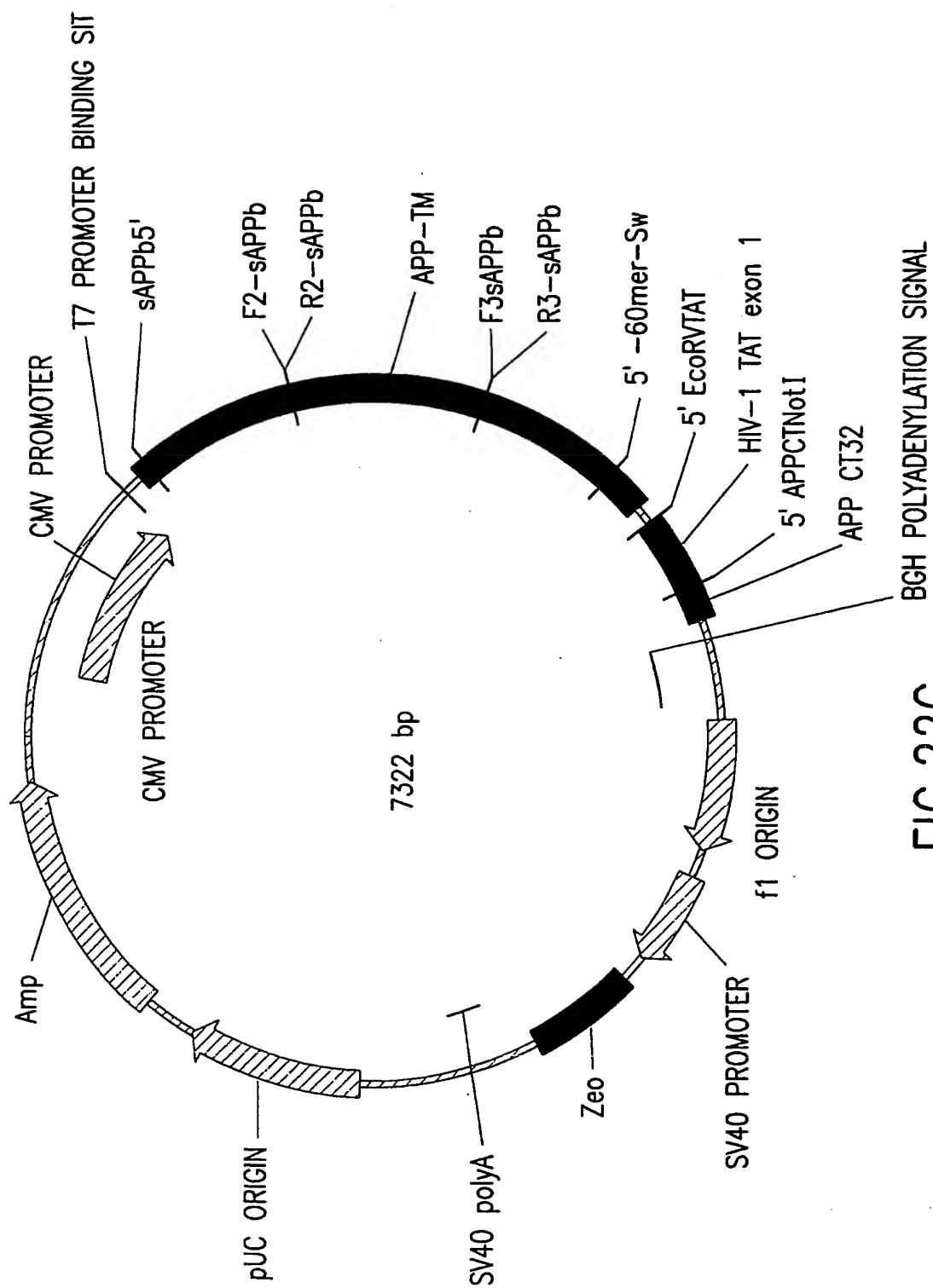


FIG.22C

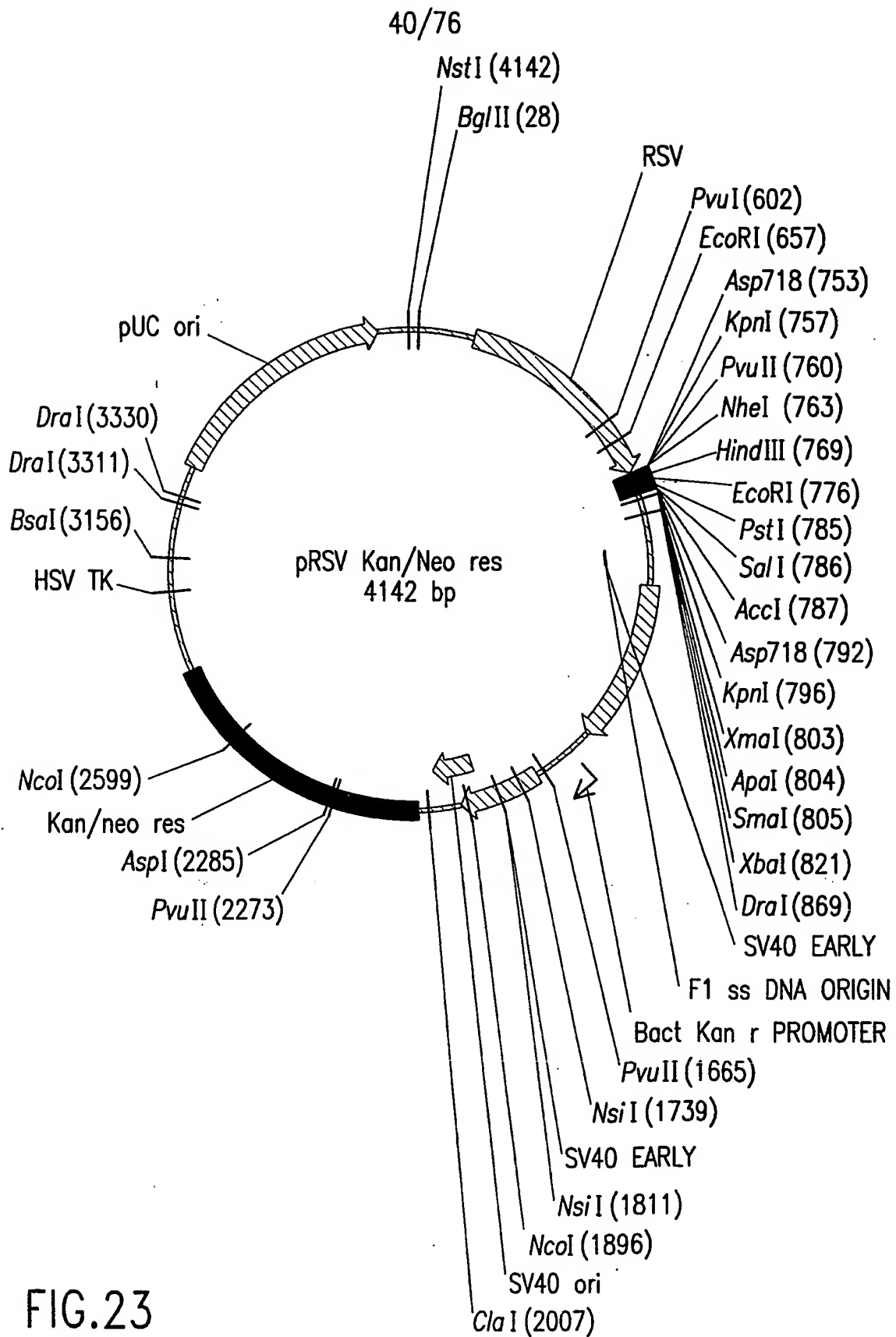


FIG.23

41/76

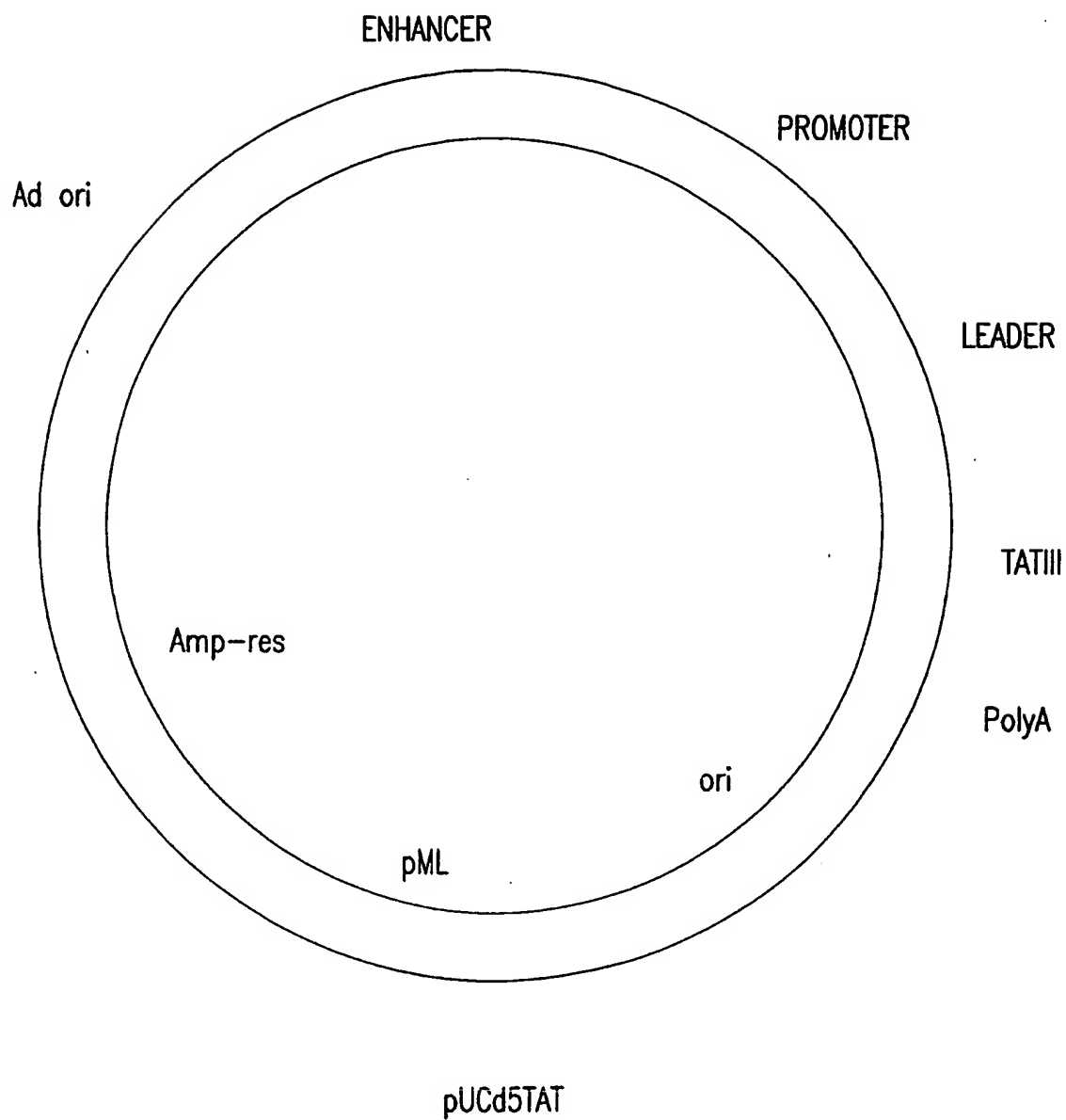


FIG.24

42/76

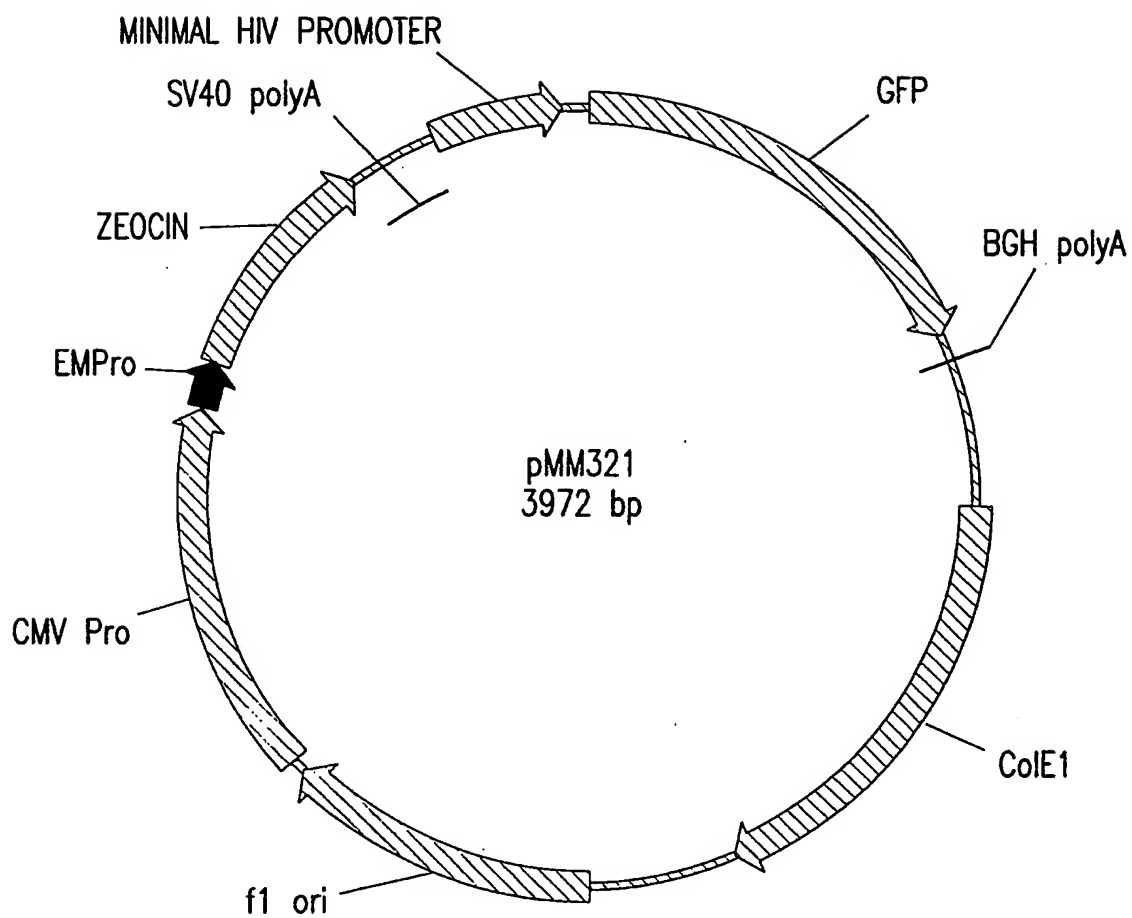


FIG.25A



43/76

(SEQ ID NO: 19 AND 20)

```

1  ATGGTGAGCA AGGGCGAGGA GCTGTTCAAC GGGGTGGTGC CCATCCTGGT
   TACCACTCGT TCCCGCTCCT CGACAAGTGG CCCCACCACG GGTAGGACCA
51  CGAGCTGGAC GGGGACGTAA ACGGCCACAA GTTCAGCGTG TCCGGCGAGG
   GCTCGACCTG CCCTGTCATT TGCCGGTGTT CAAGTCGCAC AGGCCGCTCC
101  GCGAGGGCGA TGCCACCTAC GGCAAGCTGA CCCTGAAGTT CATCTGCACC
   CGCTCCCGCT ACGGTGGATG CCGTTCGACT GGGACTTCAA GTAGACGTGG
151  ACCGGCAAGC TGCCCGTGCC CTGGCCACAC CTCGTGACCA CCTTCACCTA
   TGGCCGTTTCG ACGGGCACGG GACCGGGTGG GAGCACTGGT GGAAGTGGAT
201  CGGCGTGACG TGCTTCGCCC GCTACCCCGA CCACATGAAG CAGCACGACT
   GCCGCACGTC ACGAAGCGGG CGATGGGGCT GGTGTACTTC GTCGTGCTGA
251  TCTTCAAGTC CGCCATGCCC GAAGGCTACG TCCAGGAGCG CACCATCTTC
   AGAAGTTCAG GCGGTACGGG CTTCCGATGC AGGTCTCTCG GTGGTAGAAG
301  TTCAAGGACG ACGGCAACTA CAAGACCCGC GCCGAGGTGA AGTTCGAGGG
   AAGTTCCTGC TGCCGTTGAT GTTCTGGGCG CGGCTCCACT TCAAGCTCCC
351  CGACACCCTG GTGAACCGCA TCGAGCTGAA GGGCATCGAC TTCAAGGAGG
   GCTGTGGGAC CACTTGGCGT AGCTCGACTT CCCGTAGCTG AAGTTCCTCC
401  ACGGCAACAT CCTGGGGCAC AAGCTGGAGT ACAACTACAA CAGCCACAAG
   TGCCGTTGTA GGACCCCGTG TTCGACCTCA TGTGATGTT GTCGGTGTTC
451  GTCTATATCA CCGCCGACAA GCAGAAGAAC GGCATCAAGG TGAACITCAA
   CAGATATAGT GCGGGCTGTT CGTCTTCTTG CCGTAGTTCC ACTTGAAGTT
501  GACCCGCCAC AACATCGAGG ACGGCAGCGT GCAGCTCGCC GACCACTACC
   CTGGGCGGTG TTGTAGCTCC TGCCGTGCGA CGTCGAGCGG CTGGTGATGG
551  AGCAGAACAC CCCCATCGGC GACGGCCCCG TGCTGCTGCC CGACAACCAC
   TCGTCTTGTG GGGGTAGCCG CTGCCGGGGC ACGACGACGG GCTGTTGGTG
601  TACCTGAGCA CCCAGTCCGC CCTGAGCAAA GACCCCAACG AGAAGCGCGA
   ATGGACTCGT GGGTCAGGCG GGAATCGTTT CTGGGGTTGC TCTTCGCGCT
651  TCACATGGTC CTGCTGGAGT TCGTGACCGC CGCCGGGATC ACTCTCGGCA
   AGTGTACCAG GACGACCTCA AGCACTGGCG GCGGCCCTAG TGAGAGCCGT
701  TGGACGAGCT GTACAAGTAA CTCGAGTCTA GAGGGCCCGT TTAACCCCGC
   ACCTGCTCGA CATGTTTATT GAGCTCAGAT CTCCCAGGCA AATTGCGGCG
751  TGATCAGCCT CGACTGTGCC TTCTAGTTGC CAGCCATCTG TTGTTTGCCC
   ACTAGTCGGA GCTGACACGG AAGATCAACG GTCGGTAGAC AACAAACGGG
801  CTCCCCCGTG CCTTCCTTGA CCCTGGAAGG TGCCACTCCC ACTGTCCTTT
   GAGGGGGCAC GGAAGGAACT GGGACCTTCC ACGGTGAGGG TGACAGGAAA
851  CCTAATAAAA TGAGGAAATT GCATCGCATT GTCTGAGTAG GTGTCATTCT
   GGATTATTTT ACTCCTTTAA CGTAGCGTAA CAGACTCATC CACAGTAAGA
901  ATTCTGGGGG GTGGGGTGGG GCAGGACAGC AAGGGGGAGG ATTGGGAAGA
   TAAGACCCCC CACCCACCCC CGTCCTGTCTG TTCCCCCTCC TAACCTTTCT
951  CAATAGCAGG CATGCTGGGG ATGCGGTGGG CTCTATGGCT TCTGAGGCGG
   GTTATCGTCC GTACGACCCC TACGCCACCC GAGATACCGA AGACTCCGCC
1001  AAAGAACCAG CATGTGAGCA AAAGGCCAGC AAAAGGCCAG GAACCGTAAA
   TTTCTTGGTC GTACACTCGT TTTCCGGTCG TTTTCCGGTC CTTGGCATT
1051  AAGGCCGCGT TGCTGGCGTT TTTCCATAGG CTCCGCCCCC CTGACGAGCA
   TTCCGGCGCA ACGACCGCAA AAAGGTATCC GAGGCCGGGG GACTGCTCGT
1101  TCACAAAAAT CGACGCTCAA GTCAGAGGTG GCGAAACCCG ACAGGACTAT
   AGTGTTTTTA GCTGCGAGTT CAGTCTCCAC CGCTTTGGGC TGTCTGTATA
1151  AAAGATACCA GCGGTTTCCC CCTGGAAGCT CCCTCGTGCG CTCTCCTGTT
   TTTCTATGGT CCGCAAAGGG GGACCTTCGA GGGAGCACGC GAGAGGACAA
1201  CCGACCCTGC CGCTTACCGG ATACCTGTCC GCCTTTCTCC CTTGCGGAAG
   GGCTGGGACG GCGAATGGCC TATGGACAGG CCGAAAGAGG GAAGCCCTTC
1251  CGTGGCGCTT TCTCATAGCT CACGCTGTAG GTATCTCAGT TCGGTGTAGG
   GCACCGCGAA AGAGTATCGA GTGCGACATC CATAGAGTCA AGCCACATCC
1301  TCGTTGCTC CAAGCTGGGC TGTGTGCACG AACCCCCCGT TCAGCCCGAC
   AGCAAGCGAG GTTCGACCCG ACACACGTGC TTGGGGGGCA AGTCGGGCTG
1351  CGCTGCGCCT TATCCGGTAA CTATCGTCTT GAGTCCAACC CGGTAAGACA
   CCGACGCGGA ATAGGCCATT GATAGCAGAA CTCAGGTTGG GCCATTCTGT
1401  CGACTTATCG CCACTGGCAG CAGCCACTGG TAACAGGATT AGCAGAGCGA
   GCTGAATAGC GGTGACCGTC GTCGGTGACC ATTGTCCTAA TCCTCTCGCT

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FIG.25B

44/76

1451	GGTATGTAGG	CGGTGCTACA	GAGTTCTTGA	AGTGGTGGCC	TAACACGGC
	CCATACATCC	GCCACGATGT	CTCAAGAACT	TCACCACCGG	ATTGATGCCG
1501	TACACTAGAA	GAACAGTATT	TGGTATCTGC	GCTCTGCTGA	AGCCAGTTAC
	ATGTGATCTT	CTTGTCATAA	ACCATAGACG	CGAGACGACT	TCGGTCAATG
1551	CTTCGGA AAA	AGAGTTGGTA	GCTCTTGATC	CGGCAAACAA	ACCACCGCTG
	GAAGCCTTTT	TCTCAACCAT	CGAGAACTAG	GCCGTTTGTT	TGGTGGCGAC
1601	GTAGCGGTGG	TTTTTTTGTT	TGCAAGCAGC	AGATTACGCG	CAGAAAAAAA
	CATGCCACC	AAAAAAACAA	ACGTTTCGTCG	TCTAATGCGC	GTCTTTTTTT
1651	GGATCTCAAG	AAGATCCTTT	GATCTTTTCT	ACGGGGTCTG	ACGCTCAGTG
	CCTAGAGTTC	TTCTAGGAAA	CTAGAAAAGA	TGCCCCAGAC	TGCGAGTCAC
1701	GAACGAAAAAC	TCACGTTAAG	GGATTTTGTT	CATGACATTA	ACCTATAAAA
	CTTGCTTTTG	AGTGCAATTC	CCTAAAACCA	GTA CTGTAAT	TGGATATTTT
1751	ATAGGCGTAT	CACGAGGCC	TTTCGTCTCG	CGCGTTTCGG	TGATGACGGT
	TATCCGCATA	GTGCTCCGGG	AAAGCAGAGC	GCGCAAAGCC	ACTACTGCCA
1801	GA AAACCTCT	GACACATGCA	GCTCCCGGAG	ACGGTCACAG	CTTGTCTGTA
	CTTTTGGAGA	CTGTGTACGT	CGAGGGCCTC	TGCCAGTGTC	GAACAGACAT
1851	AGCGGATGCC	GGGAGCAGAC	AAGCCCGTCA	GGGCGCGTCA	GCGGGTGTTG
	TCGCCTACGG	CCCTCGTCTG	TTCGGGCAGT	CCGCGCAGT	CGCCACAAC
1901	GCGGGTGTCG	GGGCTGGCTT	AACTATGCGG	CATCAGAGCA	GATTGTACTG
	CGCCACAGC	CCCGACCGAA	TTGATACGCC	GTA GTCTCGT	CTAACATGAC
1951	AGAGTGCACC	ATATGCGGTG	TGAAATACCG	CACAGATGCG	TAAGGAGAAA
	TCTACGTGG	TATACGCCAC	ACTTTATGGC	GTGTCTACGC	ATTCCTCTTT
2001	ATACCGCATC	AGGACGCGCC	CTGTAGCGGC	GCATTAAGCG	CGGCGGGTGT
	TATGGCGTAG	TCCTGCGCGG	GACATCGCCG	CGTAATTCGC	GCCGCCACA
2051	GGTGGTTACG	CGCAGCGTGA	CCGCTACACT	TGCCAGCGCC	CTAGCGCCCG
	CCACCAATGC	GCGTCGCACT	GGCGATGTGA	ACGGTCGCGG	GATCGCGGGG
2101	CTCCTTTTCG	TTTTTCCCT	TCCTTTCTCG	CCACGTTTCG	CGGCTTTCC
	GAGGAAAGCG	AAAGAAGGGA	AGGAAAGAGC	GGTGCAAGCG	GCCGAAAGGG
2151	CGTCAAGCTC	TAAATCGGGG	GCTCCCTTTA	GGGTTCCGAT	TTAGTGCTTT
	GCAGTTCGAG	ATTTAGCCCC	CGAGGGAAT	CCCAAGGCTA	AATCACGAAA
2201	ACGGCACCTC	GACCCCAAAA	AACTTGATTA	GGGTGATGGT	TCACGTAGTG
	TGCGGTGGAG	CTGGGGTTTT	TTGAACTAAT	CCCCTACCA	AGTGCATCAC
2251	GGCCATCGCC	CTGATAGACG	GTTTTTCGCC	CTTTGACGTT	GGAGTCCACG
	CCGGTAGCGG	GA CTATCTGC	CAAAAAGCGG	GAAACTGCAA	CCTCAGGTGC
2301	TTCTTTTAATA	GTGGA CTCTT	GTTCCAAACT	GGAACAACAC	TCAACCCTAT
	AAGAAATTAT	CACCTGAGAA	CAAGGTTTGA	CCTTGTTGTG	AGTTGGGATA
2351	CTCGGTCTAT	TC TTTTGATT	TATAAGGGAT	TTTGCCGATT	TCGGCCTATT
	GAGCCAGATA	AGAAAACTAA	ATATTCCCTA	AAACGGCTAA	AGCCGGATAA
2401	GGTTAAAAAA	TGAGCTGATT	TAACAAAAAT	TTAACGCGAA	TTTTAACAAA
	CCAA TTTTTT	ACTCGACTAA	ATTGTTTTTA	AATTGCGCTT	AAAATTGTTT
2451	ATATTAACGC	TTACAATTTT	CATTGCGCAT	TCAGGCTGAA	CTAGATCTAG
	TATAATTGCG	AATGTTAAAG	GTAAGCGGTA	AGTCCGACTT	GATCTAGATC
2501	AGTCCGTTAC	ATAACTTACG	GTA AATGGCC	CGCCTGGCTG	ACCGCCCAAC
	TCAGGCAATG	TATTGAATGC	CATTTACCGG	GCGGACCGAC	TGGCGGGTTG
2551	GACCCCGGCC	CATTGACGTC	AATAATGACG	TATGTTCCCA	TAGTAACGCC
	CTGGGGGCGG	GTA ACTGCAG	TTATTACTGC	ATACAAGGGT	ATCATTGCGG
2601	AATAGGGACT	TTCCATTGAC	GTCAATGGGT	GGAGTATTTA	CGGTAAACTG
	TTATCCCTGA	AAGGTA ACTG	CAGTTACCCA	CCTCATAAAT	GCCATTTGAC
2651	CCCACTTGGC	AGTACATCAA	GTGTATCATA	TGCCAAGTAC	GCCCCCTATT
	GGGTGAACCG	TCATGTAGTT	CACATAGTAT	ACGGTTCATG	CGGGGGATAA
2701	GACGTCAATG	ACGGTAAATG	GCCGCTGCTG	CATTATGCCC	AGTACATGAC
	CTGCAGTTAC	TGCCATTTAC	CGGGCGGACC	GTAATACGGG	TCATGTACTG
2751	CTTATGGGAC	TTTCCTACTT	GGCAGTACAT	CTACGTATTA	GTCATCGCTA
	GAATACCTTG	AAAGGATGAA	CCGT CATGTA	GATGCATAAT	CAGTAGCGAT
2801	TTACCATGGT	GATGCGGTTT	TGGCAGTACA	TCAATGGGCG	TGGATAGCGG
	AATGGTACCA	CTACGCCAAA	ACCGTCATGT	AGTTACCCGC	ACCTATCGCC
2851	TTTGACTCAC	GGGGATTTCC	AAGTCTCCAC	CCCATTGACG	TCAATGGGAG
	AAACTGAGTG	CCCCTAAAGG	TTCAGAGGTG	GGGTA ACTGC	AGTTACCCTC

FIG.25C

45/76

2901 TTTGTTTTGG CACCAAAATC AACGGGACTT TCCAAAATGT CGTAACAACCT  
AAACAAAACC GTGGTTTTAG TTGCCCTGAA AGGTTTTTACA GCATTGTTGA  
2951 CCGCCCCATT GACGCAAATG GCGGGTAGGC GTGTACGGTG GGAGGTCTAT  
GGCGGGGTAA CTGCGTTTAC CCGCCATCCG CACATGCCAC CCTCCAGATA  
3001 ATAAGCAGAG CTCGTTTAGT GAACCGTCAG ATCGCCTGGA GACGCCATCC  
TATTCGTCTC GAGCAAATCA CTTGGCAGTC TAGCGGACCT CTGCGGTAGG  
3051 ACGCTGTTTT GACCTCCATA GAAGACACCG GGACCGATCC AGCCTCCGCG  
TGCGACAAAA CTGGAGGTAT CTTCTGTGGC CCTGGCTAGG TCGGAGGCGC  
3101 GCCGGGAACG GTGCATTGGA ACGGACCGTG TTGACAATTA ATCATCGGCA  
CGGCCCTTGC CACGTAACCT TGCCTGGCAC AACTGTTAAT TAGTAGCCGT  
3151 TAGTATATCG GCATAGTATA ATACGACAAG GTGAGGAACT AAACCATGGC  
ATCATATAGC CGTATCATAT TATGCTGTTT CACTCCTTGA TTTGGTACCG  
3201 CAAGTTGACC AGTGCCGTTT CCGTGCTCAC CGCGCGCGAC GTCGCCGGAG  
GTTCAACTGG TCACGGCAAG GCCACGAGTG GCGCGCGCTG CAGCGGCCCTC  
3251 CGGTCGAGTT CTGGACCGAC CGGCTCGGGT TCTCCCGGGA CTTCTGTGGG  
GCCGCTCAA GACCTGGCTG GCCGAGCCCA AGAGGGCCCT GAAGCACCTC  
3301 GACGACTTCG CCGGTGTGGT CCGGGACGAC GTGACCCTGT TCATCAGCGC  
CTGCTGAAGC GGCCACACCA GGCCCTGCTG CACTGGGACA AGTAGTCGCG  
3351 GGTCCAGGAC CAGGTGGTGC CGGACAACAC CCTGGCCTGG GTGTGGGTGC  
CCAGGTCTTG GTCCACCACG GCCTGTTGTG GGACCGGACC CACACCCACG  
3401 GCCGCCCTGA CGAGCTGTAC GCCGAGTGGT CGGAGGTCGT GTCCACGAAC  
CGCCGGACCT GCTCGACATG CGGCTCACCA GCCTCCAGCA CAGGTGCTTG  
3451 TTCCGGGACG CCTCCGGGCC GGCCATGACC GAGATCGGCG AGCAGCCGTG  
AAGGCCCTGC GGAGGCCCGG CCGGTACTGG CTCTAGCCGC TCGTCGGCAC  
3501 GGGGCGGGAG TTCGCCCTGC GCGACCCGGC CGGCAACTGC GTGCACTTCG  
CCCCGCCCTC AAGCGGGACG CGCTGGGCCG GCCGTTGACG CACGTGAAGC  
3551 TGGCCGAGGA GCAGGACTGA CACTCGACCT CGAAACTTGT TTATTGCAGC  
ACCGGCTCCT CGTCTGACT GTGAGCTGGA GCTTTGAACA AATAACGTCG  
3601 TTATAATGGT TACAAATAAA GCAATAGCAT CACAAATTTT ACAAATAAAG  
AATATTACCA ATGTTTATTT CGTTATCGTA GTGTTTAAAG TGTATTATTC  
3651 CATTTTTTTC ACTGCATTCT AGTTGTGGTT TGTCCAACT CATCAATGTA  
GTAAAAAAG TGACGTAAGA TCAACACCAA ACAGGTTTGA GTAGTTACAT  
3701 TCTTATCATG TCTGGATCGA TACTTCAAGA ACTGCTGACA TCGAGCTTGC  
AGAATAGTAC AGACCTAGCT ATGAAGTTCT TGACGACTGT AGCTCGAAGC  
3751 TACAAGGGAC TTTCCGCTGG GGACTTTCCA GGGAGGCGTG GCCTGGGCGG  
ATGTTCCCTG AAAGGCGACC CCTGAAAGGT CCCTCCGCAC CGGACCCGCC  
3801 GACTGGGGAG TGGCGAGCCC TCAGATCCTG CATATAAGCA GCTGCTTTTT  
CTGACCCCTC ACCGCTCGGG AGTCTAGGAC GTATATTCTG CGACGAAAAA  
3851 GCCTGTACTG GGTCTCTCTG GTTAGACCAG ATCTGAGCCT GGGAGCTCTC  
CGGACATGAC CCAGAGAGAC CAATCTGGTC TAGACTCGGA CCCTCGAGAG  
3901 TGGCTAACTA GGAACCCAC TGCTTAAGCC TCAATAAAGC TTGGTACCGA  
ACCGATTGAT CCCTTGGGTG ACGAATTCGG AGTTATTTCT AACCATGGCT  
3951 GCTCGGATCC GAATTCGCCA CC  
CGAGCCTAGG CTTAAGCGGT GG

FIG.25D

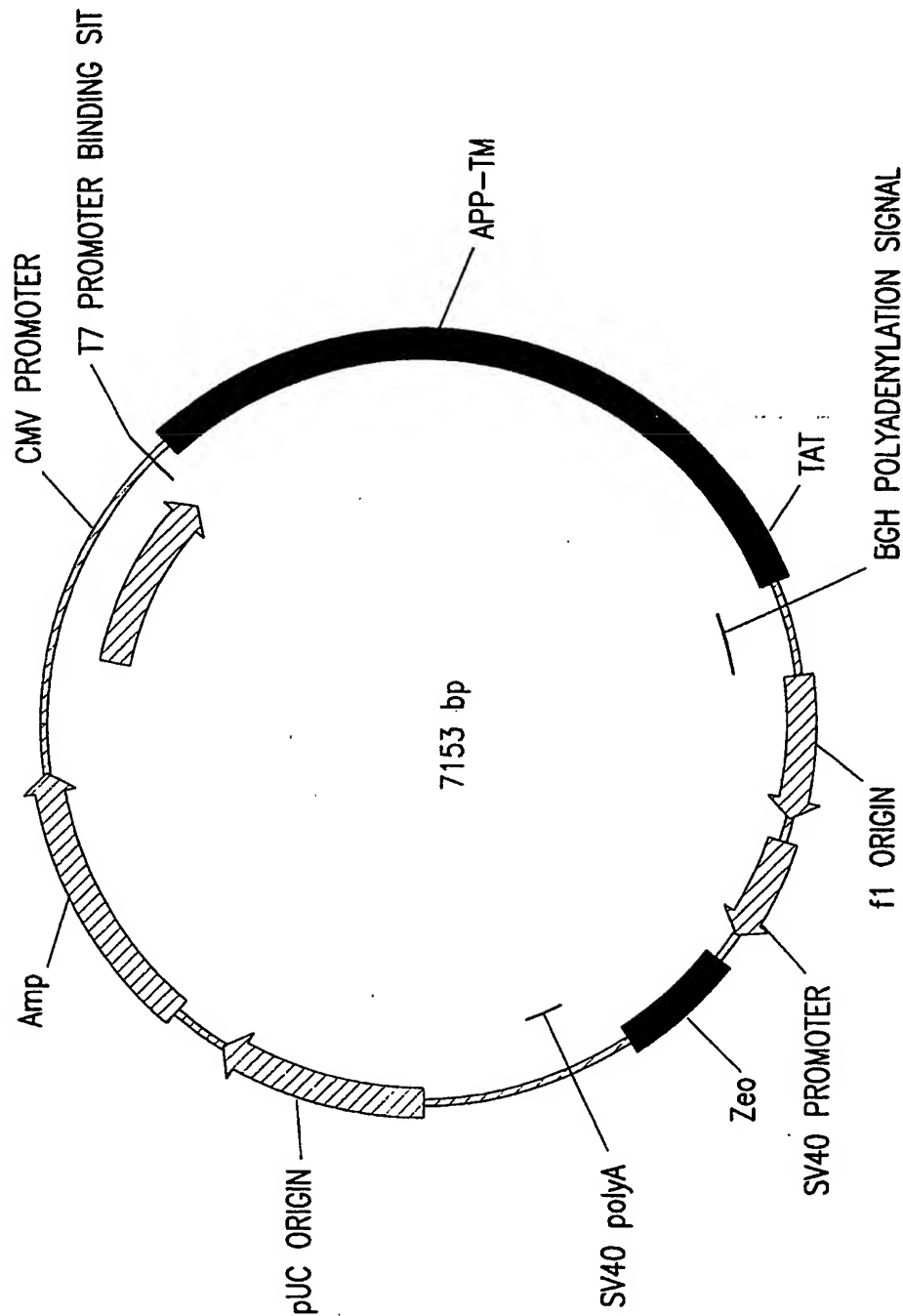


FIG.26A

47/76

(SEQ ID NO: 21 AND 22)

1	GACGGATCGG	GAGATCTCCC	GATCCCCTAT	GGTCGACTCT	CAGTACAATC
	CTGCCTAGCC	CTCTAGAGGG	CTAGGGGATA	CCAGCTGAGA	GTCATGTTAG
51	TGCTCTGATG	CCGCATAGTT	AAGCCAGTAT	CTGCTCCCTG	CTTGTGTGTT
	ACGAGACTAC	GGCGTATCAA	TTCGGTCATA	GACGAGGGAC	GAACACACAA
101	GGAGGTCGCT	GAGTAGTGCG	CGAGCAAAAT	TTAAGCTACA	ACAAGGCAAG
	CCTCCAGCGA	CTCATCACGC	GCTCGTTTTA	AATTCGATGT	TGTTCCGTTT
151	GCTTGACCGA	CAATTGCATG	AAGAATCTGC	TTAGGGTTAG	GCGTTTTGCG
	CGAACTGGCT	GTTAACGTAC	TTCTTAGACG	AATCCCAATC	CGCAAAACGC
201	CTGCTTCGCG	ATGTACGGGC	CAGATATACG	CGTTGACATT	GATTATTGAC
	GACGAAGCGC	TACATGCCCC	GTCTATATGC	GCAACTGTAA	CTAATAACTG
251	TAGTTATTAA	TAGTAATCAA	TTACGGGGTC	ATTAGTTCAT	AGCCCATATA
	ATCAATAATT	ATCATTAGTT	AATGCCCCAG	TAATCAAGTA	TCGGGTATAT
301	TGGAGTTCGG	CGTTACATAA	CTTACGGTAA	ATGGCCCCGC	TGGCTGACCG
	ACCTCAAGGC	GCAATGTATT	GAATGCCATT	TACCGGGCGG	ACCGACTGGC
351	CCCAACGACC	CCCGCCCAT	GACGTCAATA	ATGACGTATG	TTCCCATAGT
	GGGTTGCTGG	GGGCGGGTAA	CTGCAGTTAT	TACTGCATAC	AAGGGTATCA
401	AACGCCAATA	GGGACTTTCC	ATTGACGTCA	ATGGGTGGAC	TATTTACGGT
	TTGCGGTTAT	CCCTGAAAGG	TAAGTGCAGT	TACCCACCTG	ATAAATGCCA
451	AAACTGCCCC	CTTGGCAGTA	CATCAAGTGT	ATCATATGCC	AAGTACGCC
	TTTGACGGGT	GAACCGTCAT	GTAGTTTACA	TAGTATACGG	TTCATGCGGG
501	CCTATTGACG	TCAATGACGG	TAAATGGCCC	GCCTGGCATT	ATGCCAGTA
	GGATAACTGC	AGTTACTGCC	ATTTACGGGG	CGGACCGTAA	TACGGGTCAT
551	CATGACCTTA	TGGGACTTTC	CTACTTGGCA	GTACATCTAC	GTATTAGTCA
	GTAAGTGAAT	ACCCTGAAAG	GATGAACCGT	CATGTAGATG	CATAATCAGT
601	TCGCTATTAC	CATGGTGATG	CGGTTTTGGC	AGTACATCAA	TGGGCGTGGA
	AGCGATAATG	GTACCACTAC	GCCAAAACCG	TCATGTAGTT	ACCCGCACCT
651	TAGCGGTTTG	ACTCACGGGG	ATTTCCAAGT	CTCCACCCCA	TTGACGTCAA
	ATCGCCAAAC	TGAGTGCCCC	TAAAGGTTCA	GAGGTGGGGT	AACTGCAGTT
701	TGGGAGTTTG	TTTTGGCACC	AAAATCAACG	GGACTTTCCA	AAATGTCGTA
	ACCCTCAAAC	AAAACCGTGG	TTTTAGTTGC	CCTGAAAGGT	TTTACAGCAT
751	ACAACTCCGC	CCCATTGACG	CAAATGGGCG	GTAGGCGTGT	ACGGTGGGAG
	TGTTGAGGCG	GGGTAAGTGC	GTTTACCCGC	CATCCGCACA	TGCCACCCTC
801	GTCTATATAA	GCAGAGCTCT	CTGGCTAACT	AGAGAACCCA	CTGCTTACTG
	CAGATATATT	CGTCTCGAGA	GACCGATTGA	TCTCTTGGGT	GACGAATGAC
851	GCTTATCGAA	ATTAATACGA	CTCACTATAG	GGAGACCCAA	GCTGGCTAGC
	CGAATAGCTT	TAATTATGCT	GAGTGATATC	CCTCTGGGTT	CGACCGATCG
901	GTTTAAACTT	AAGCTTCCCC	GCGCAGGGTC	GCGATGCTGC	CCGGTTTGGC
	CAAATTTGAA	TTCGAAGGGG	CGCGTCCAG	CGCTACGACG	GGCCAAACCG
951	ACTGCTCCTG	CTGGCCGCCT	GGACGGCTCG	GGCGCTGGAG	GTACCCACTG
	TGACGAGGAC	GACCGGCGGA	CCTGCCGAGC	CCGCGACCTC	CATGGGTGAC
1001	ATGGTAATGC	TGGCCTGCTG	GCTGAACCCC	AGATTGCCAT	GTTCTGTGGC
	TACCATTACG	ACCGGACGAC	CGACTTGGGG	TCTAACGGTA	CAAGACACCG
1051	AGACTGAACA	TGCACATGAA	TGTCCAGAAT	GGGAAGTGGG	ATTCAGATCC
	TCTGACTTGT	ACGTGTACTT	ACAGGTCTTA	CCCTTCACCC	TAAGTCTAGG
1101	ATCAGGGACC	AAAACCTGCA	TTGATACCAA	GGAAGGCATC	CTGCAGTATT
	TAGTCCCTGG	TTTTGGACGT	AACTATGGTT	CCTTCCGTAG	GACGTCATAA
1151	GCCAAAGAAGT	CTACCCTGAA	CTGCAGATCA	CCAATGTGGT	AGAAGCCAAC
	CGGTCTCTTCA	GATGGGACTT	GACGTCTAGT	GGTTACACCA	TCTTCGGTTG
1201	CAACCAAGTGA	CCATCCAGAA	CTGGTGCAAG	CGGGGCCGCA	AGCAGTGCAA
	GTTGGTCACT	GGTAGGTCTT	GACCACGTTT	GCCCCGGCGT	TCGTACAGTT
1251	GACCCATCCC	CACTTTGTGA	TTCCCTACCG	CTGCTTAGTT	GGTGAGTTTA
	CTGGGTAGGG	GTGAAACACT	AAGGGATGGC	GACGAATCAA	CCACTCAAAT
1301	TAAGTGATGC	CCTTCTCGTT	CCTGACAAGT	GCAAATCTT	ACACCAGGAG
	ATTCACTACG	GGAAGAGCAA	GGACTGTTCA	CGTTTAAGAA	TGTGGTCCTC
1351	AGGATGGATG	TTTGCGAAAC	TCATCTTCAC	TGGCACACCG	TCGCCAAAGA
	TCCTACCTAC	AAACGCTTTG	AGTAGAAGTG	ACCGTGTGGC	AGCGGTTTCT

FIG.26B

48/76

1401	GACATGCAGT	GAGAAGAGTA	CCAACCTTGCA	TGACTACGGC	ATGTTGCTGC
	CTGTACGTCA	CTCTTCTCAT	GGTTGAACGT	ACTGATGCCG	TACAACGACG
1451	CCTGCGGAAT	TGACAAGTTC	CGAGGGGTAG	AGTTTGTGTG	TTGCCCACTG
	GGACGCCTTA	ACTGTTCAAG	GCTCCCATC	TCAAACACAC	AACGGGTGAC
1501	GCTGAAGAAA	GTGACAATGT	GGATTCTGCT	GATGCGGAGG	AGGATGACTC
	CGACTTCTTT	CACTGTTACA	CCTAAGACGA	CTACGCCTCC	TCCTACTGAG
1551	GGATGTCTGG	TGGGGCGGAG	CAGACACAGA	CTATGCAGAT	GGGAGTGAAG
	CCTACAGACC	ACCCCGCCTC	GTCTGTGTCT	GATACGTCTA	CCCTCACTTC
1601	ACAAAGTAGT	AGAAGTAGCA	GAGGAGGAAG	AAGTGGCTGA	GGTGAAGAA
	TGTTTTATCA	TCTTCATCGT	CTCCTCCTTC	TTCACCGACT	CCACCTTCTT
1651	GAAGAAGCCG	ATGATGACGA	GGACGATGAG	GATGGTGATG	AGGTAGAGGA
	CTTCTTCGGC	TACTACTGCT	CCTGCTACTC	CTACCACTAC	TCCATCTCCT
1701	AGAGGCTGAG	GAACCCTACG	AAGAAGCCAC	AGAGAGAACC	ACCAGCATTG
	TCTCCGACTC	CTTGGGATGC	TTCTTCGGTG	TCTCTTTGG	TGGTCGTAAC
1751	CCACCACCA	CACCACCACC	ACAGAGTCTG	TGGAAGAGGT	GGTTCGAGTT
	GGTGGTGGTG	GTGGTGGTGG	TGTCTCAGAC	ACCTTCTCCA	CCAAGCTCAA
1801	CCTACAACAG	CAGCCAGTAC	CCCTGATGCC	GTTGACAAGT	ATCTCGAGAC
	GGATGTTGTC	GTCGGTCATG	GGGACTACGG	CAACTGTTCA	TAGAGCTCTG
1851	ACCTGGGGAT	GAGAATGAAC	ATGCCCATTT	CCAGAAAGCC	AAAGAGAGGC
	TGGACCCCTA	CTCTTACTTG	TACGGGTAAA	GGTCTTTCGG	TTTCTCTCCG
1901	TTGAGGCCAA	GCACCGAGAG	AGAATGTCCC	AGGTCATGAG	AGAATGGGAA
	AACTCCGGTT	CGTGGCTCTC	TCTTACAGGG	TCCAGTACTC	TCTTACCCTT
1951	GAGGCAGAAC	GTCAAGCAAA	GAACCTGCCT	AAAGCTGATA	AGAAGGCAGT
	CTCCGTCTTG	CAGTTCGTTT	CTTGAACGGA	TTTCGACTAT	TCTTCCGTCA
2001	TATCCAGCAT	TTCCAGGAGA	AAGTGGAATC	TTTGGAACAG	GAAGCAGCCA
	ATAGGTGCTA	AAGGTCTCT	TTCACCTTAG	AAACCTTGTC	CTTCGTGGT
2051	ACGAGAGACA	GCAGCTGGTG	GAGACACACA	TGGCCAGAGT	GGAAGCCATG
	TGCTCTCTGT	CGTCGACCAC	CTCTGTGTGT	ACCGGTCTCA	CCTTCGGTAC
2101	CTCAATGACC	GCCGCCGCCCT	GGCCCTGGAG	AACTACATCA	CCGCTCTGCA
	GAGTTACTGG	CGGCGGCGGA	CCGGGACCTC	TTGATGTAGT	GGCGAGACGT
2151	GGCTGTTCT	CCTCGGCCTC	GTCACGTGTT	CAATATGCTA	AAGAAGTATG
	CCGACAAGGA	GGAGCCGGAG	CAGTGCACAA	GTTATACGAT	TTCTTCATAC
2201	TCCGCGCAGA	ACAGAAGGAC	AGACAGCACA	CCCTAAAGCA	TTTCGAGCAT
	AGGCGCGTCT	TGTCTTCTG	TCTGTCTGT	GGGATTTCTG	AAAGCTCGTA
2251	GTGCGCATGG	TGGATCCCAA	GAAAGCCGCT	CAGATCCGGT	CCCAGTTTAT
	CACGCGTACC	ACCTAGGGTT	CTTTCGGCGA	GTCTAGGCCA	GGGTCCAATA
2301	GACACACCTC	CGTGTGATTT	ATGAGCGCAT	GAATCAGTCT	CTCTCCCTGC
	CTGTGTGGAG	GCACACTAAA	TACTCGCGTA	CTTAGTCAGA	GAGAGGGACG
2351	TCTACAACGT	GCCTGCAGTG	GCCGAGGAGA	TTCAGGATGA	AGTTGATGAG
	AGATGTTGCA	CGGACGTCAC	CGGCTCCTCT	AAGTCCTACT	TCAACTACTC
2401	CTGCTTCAGA	AAGAGCAAAA	CTATTCAGAT	GACGCTTTGG	CCAACATGAT
	GACGAAGTCT	TTCTCGTTTT	GATAAGTCTA	CTGCAGAAC	GGTTGTACTA
2451	TAGTGAACCA	AGGATCAGTT	ACGGAACGA	TGCTCTCATG	CCATCTTTGA
	ATCACTTGGT	TCCTAGTCAA	TGCCCTTGCT	ACGAGAGTAC	GGTAGAAACT
2501	CCGAAACGAA	AACCACCGTG	GAGCTCCTTC	CCGTGAATGG	AGAGTTCAGC
	GGCTTTGCTT	TTGGTGGCAC	CTCGAGGAAG	GGCACTTACC	TCTCAAGTCG
2551	CTGGACGATC	TCCAGCCGTG	GCATTCTTTT	GGGGCTGACT	CTGTGCCAGC
	GACCTGCTAG	AGGTGCGGAC	CGTAAGAAAA	CCCCGACTGA	GACACGGTCTG
2601	CAACACAGAA	AACGAAGTTG	AGCCTGTTGA	TGCCCGCCCT	GCTGCCGACC
	GTTGTGTCTT	TTGCTTCAAC	TGGGACAAC	ACGGGCGGGA	CGACGGCTGG
2651	GAGGACTGAC	CACTCGACCA	GGTTCTGGGT	TGACAAATAT	CAAGACGGAG
	CTCCTGACTG	GTGAGCTGGT	CCAAGACCCA	ACTGTTTATA	GTTCTGCCTC
2701	GAGATCTCTG	AAGTGAATCT	AGATGCAGAA	TTCCGACATG	ACTCAGGATA
	CTCTAGAGAC	TTCACCTAGA	TCTACGTCTT	AAGGCTGTAC	TGAGTCCTAT
2751	TGAAGTTCAT	CATCAAAAAT	TGGTGTCTT	TGCAGAAGAT	GTGGGTTCAA
	ACTTCAAGTA	GTAGTTTTTA	ACCACAAGAA	ACGTCTTCTA	CACCCAAGTT
2801	ACAAAGGTGC	AATCATTGGA	CTCATGGTGG	GCGGTGTTGT	CATAGCGACA
	TGTTTCCACG	TAGTAACCT	GAGTACCACC	CGCCACAACA	GTATCGCTGT

FIG.26C

49/76

2851	GTGATCGTCA	TCACCTTGGT	GATGCTGAAG	AAGAAAGATA	TCATGGAGCC
	CACTAGCAGT	AGTGGAACCA	CTACGACTTC	TTCTTTCTAT	AGTACCTCGG
2901	AGTAGATCCT	AGACTAGAGC	CCTGGAAGCA	TCCAGGAAGT	CAGCCTAAAA
	TCATCTAGGA	TCTGATCTCG	GGACCTTCGT	AGGTCCTTCA	GTCGGATTTT
2951	CTGCTTGATC	CAATTGCTAT	TGTAAAAAGT	GTTGCTTTCA	TTGCCAAGTT
	GACGAACATG	GTTAACGATA	ACATTTTTCA	CAACGAAAGT	AACGGTTCAA
3001	TGTTTTATGA	CAAAAGCCTT	AGGCATCTCC	TATGGCAGGA	AGAAGCGGAG
	ACAAAGTACT	GTTTTTCGGAA	TCCGTAGAGG	ATACCGTCCT	TCTTCGCCTC
3051	ACAGCGACGA	AGAGCTCATC	AGAACAGTCA	GACTCATCAA	GCTTCTCTAT
	TGTCGCTGCT	TCTCGAGTAG	TCTTGTCAGT	CTGAGTAGTT	CGAAGAGATA
3101	CAAAGCAGTA	AGTAGGCGGC	CGCTCGAGTC	TAGAGGGCCC	GTTTAAACCC
	GTTTCGTCAT	TCATCCGCCG	GCGAGCTCAG	ATCTCCCGGG	CAAATTTGGG
3151	GCTGATCAGC	CTCGACTGTG	CCTTCTAGTT	GCCAGCCATC	TGTTGTTTGC
	CGACTAGTCG	GAGCTGACAC	GGAAGATCAA	CGGTGCGTAG	ACAACAAACG
3201	CCCTCCCCCG	TGCCCTTCTT	GACCTTGGA	GGTGCCACTC	CCACTGTCTC
	GGGAGGGGGC	ACGGAAGGAA	CTGGGACCTT	CCACGGTGAG	GGTGACAGGA
3251	TTCTTAATAA	AATGAGGAAA	TTGCATCGCA	TTGTCTGAGT	AGGTGTCATT
	AAGGATTATT	TACTCCTTT	AACGTAGCGT	AACAGACTCA	TCCACAGTAA
3301	CTATTCTGGG	GGGTGGGGTG	GGGCAGGACA	GCAAGGGGGA	GGATTGGGAA
	GATAAGACCC	CCCACCCAC	CCCGTCCTGT	CGTTCCCCCT	CCTAACCTT
3351	GACAATAGCA	GGCATGCTGG	GGATGCGGTG	GGCTCTATGG	CTTCTGAGGC
	CTGTTATCGT	CCGTACGACC	CCTACGCCAC	CCGAGATACC	GAAGACTCCG
3401	GGAAAGAACC	AGCTGGGGCT	CTAGGGGGTA	TCCCCACGCG	CCCTGTAGCG
	CCTTTCTTGG	TCGACCCCGA	GATCCCCCAT	AGGGGTGCGC	GGGACATCGC
3451	GGCGATTAAAG	CGCGGCGGGT	GTGGTGGTTA	CGCGCAGCGT	GACCGCTACA
	CGCGTAATTC	GCGCCGCCCA	CACCACCAAT	GCGCGTCGCA	CTGGCGATGT
3501	CTTGCCAGCG	CCCTAGCGCC	CGCTCCTTTC	GCTTTCTTCC	CTTCCTTTCT
	GAACGGTCGC	GGGATCGCGG	GCGAGGAAAG	CGAAAGAAGG	GAAGGAAAGA
3551	CGCCACGTTT	GCCGGCTTTC	CCCGTCAAGC	TCTAAATCGG	GGCATCCCTT
	GCGGTGCAAG	CGGCCGAAAG	GGGCAGTTCG	AGATTTAGCC	CCGTAGGGAA
3601	TAGGGTTCGG	ATTTAGTGCT	TTACGGCACC	TCGACCCCAA	AAAACCTGAT
	ATCCCAAGGC	TAAATCACGA	AATGCCGTGG	AGCTGGGGTT	TTTTGAACCTA
3651	TAGGGTGATG	GTTACGCTAG	TGGGCCATCG	CCCTGATAGA	CGGTTTTTCG
	ATCCCACTAC	CAAGTGCATC	ACCCGGTAGC	GGGACTATCT	GCCAAAAAGC
3701	CCCTTTGACG	TTGGAGTCCA	CGTTCTTTAA	TAGTGGACTC	TTGTTCCAAA
	GGGAAGCTGC	AACCTCAGGT	GCAAGAAATT	ATCACCTGAG	AACAAGGTTT
3751	CTGGAACAAC	ACTCAACCTT	ATCTCGGTCT	ATTCTTTTGA	TTTATAAGGG
	GACCTTGTTG	TGAGTTGGGA	TAGAGCCAGA	TAAGAAAAC	AAATATTCCC
3801	ATTTTGGGGA	TTTCGGCCTA	TTGGTTAAAA	AATGAGCTGA	TTTAACAAAA
	TAAACCCCT	AAAGCCGGAT	AACCAATTTT	TACTCGACT	AAATTGTTTT
3851	ATTTAACGCG	AATTAATTCT	GTGGAATGTG	TGTCAGTTAG	GGTGTGAAAA
	TAAATTGCGC	TTAATTAAGA	CACCTTACAC	ACAGTCAATC	CCACACCTTT
3901	GTCCCAGGC	TCCCAGGCA	GGCAGAAGTA	TGCAAAGCAT	GCATCTCAAT
	CAGGGGTCCG	AGGGGTCCGT	CCGTCTTCAT	ACGTTTCGTA	CGTAGAGTTA
3951	TAGTCAGCAA	CCAGGTGTGG	AAAGTCCCCA	GGCTCCCCAG	CAGGCAGAAG
	ATCAGTCGTT	GGTCCACACC	TTTCAGGGGT	CCGAGGGGTC	GTCCGTCTTC
4001	TATGCAAAGC	ATGCATCTCA	ATTAGTCAGC	AACCATAGTC	CCGCCCTTAA
	ATACGTTTCG	TACGTAGAGT	TAATCAGTCG	TTGGTATCAG	GGCGGGGATT
4051	CTCCGCCCAT	CCCGCCCTTA	ACTCCGCCCA	GTTCCGCCCA	TTCTCCGCC
	GAGGCGGGTA	GGGCGGGGAT	TGAGGCGGGT	CAAGGCGGGT	AAGAGGCGGG
4101	CATGGCTGAC	TAATTTTTTT	TATTTATGCA	GAGGCCGAGG	CCGCCTCTGC
	GTACCGACTG	ATTAAAAAAA	ATAAATACGT	CTCCGGCTCC	GGCGGAGACG
4151	CTCTGAGCTA	TTCCAGAAGT	AGTGAGGAGG	CTTTTTTGGA	GGCCTAGGCT
	GAGACTCGAT	AAGGTCTTCA	TCACTCTTCC	GAACAAACCT	CCGATCCGA
4201	TTTGCAAAAA	GCTCCCGGGA	GCTTGATAT	CCATTTTCGG	ATCTGATCAG
	AAACGTTTTT	CGAGGGCCCT	CGAACATATA	GGTAAAAGCC	TAGACTAGTC

FIG.26D

50/76

4251	CACGTGTTGA	CAATTAATCA	TCGGCATAGT	ATATCGGCAT	AGTATAATAC
	GTGCACAAC	GTTAATTAGT	AGCCGTATCA	TATAGCCGTA	TCATATTATG
4301	GACAAGGTGA	GGAACATAAC	CATGGCCAAG	TTGACCAAGT	CCGTTCCGGT
	CTGTTCCACT	CCTTGATTG	GTACCGGTTT	AACTGGTCAC	GGCAAGGCCA
4351	GCTCACCGCG	CGCGACGTCG	CCGGAGCGGT	CGAGTTCTGG	ACCGACCGGC
	CGAGTGGCGC	GCGCTGCAGC	GGCCTCGCCA	GCTCAAGACC	TGGCTGGCCG
4401	TCGGGTTCTC	CCGGGACTTC	GTGGAGGACG	ACTTCGCCGG	TGTGGTCCGG
	AGCCCAAGAG	GGCCCTGAAG	CACCTCCTGC	TGAAGCGGCC	ACACCAGGCC
4451	GACGACGTGA	CCCTGTTTAT	CAGCGCGGTC	CAGGACCAGG	TGGTGCCGGA
	CTGCTGCACT	GGGACAAGTA	GTGCGGCCAG	GTCCTGGTCC	ACCACGGCCT
4501	CAACACCCTG	GCCTGGGTGT	GGGTGCGCGG	CCTGGACGAG	CTGTACGCCG
	GTTGTGGGAC	CGGACCCACA	CCCACGCGCC	GGACCTGCTC	GACATGCGGC
4551	AGTGGTCGGA	GGTCGTGTCC	ACGAACCTCC	GGGACGCCTC	CGGGCCGGCC
	TCACCAGCCT	CCAGCACAGG	TGCTTGAAGG	CCCTGCGGAG	GCCCGGCCGG
4601	ATGACCGAGA	TCGGCGAGCA	GCCGTGGGGG	CGGGAGTTTC	CCCTGCGCGA
	TACTGGCTCT	AGCCGCTCGT	CGGCACCCCC	GCCCTCAAGC	GGGACGCGCT
4651	CCCGGCCGGC	AACTGCGTGC	ACTTCGTGGC	CGAGGAGCAG	GACTGACACG
	GGGCCGGCCG	TTGACGCACG	TGAAGCACCG	GCTCCTCGTC	CTGACTGTGC
4701	TGCTACGAGA	TTTCGATTCC	ACCGCCGCTC	TCTATGAAAG	GTTGGGCTTC
	ACGATGCTCT	AAAGCTAAGG	TGGCGGCGGA	AGATACTTTC	CAACCCGAAG
4751	GGAAATCGTT	TCCGGGACGC	CGGCTGGATG	ATCCTCCAGC	GCGGGGATCT
	CCTTAGCAAA	AGGCCCTGCG	GCCGACCTAC	TAGGAGGTTC	CGCCCTAGA
4801	CATGCTGGAG	TTCTTCGCCC	ACCCCAACTT	GTTTATTGCA	GCTTATAATG
	GTACGACCTC	AAGAAGCGGG	TGGGGTTGAA	CAAATAACGT	CGAATATTAC
4851	GTTACAAATA	AAGCAATAGC	ATCACAAATT	TCACAAATAA	AGCATTTTTT
	CAATGTTTAT	TTCTGTTATC	TAGTGTTTAA	AGTGTTTATT	TCGTAAAAAA
4901	TCAGTGCAAT	CTAGTTGTGG	TTTGTCCTAA	CTCATCAATG	TATCTTATCA
	AGTGAGCTAA	GATCAACACC	AAACAGGTTT	GAGTAGTTAC	ATAGAATAGT
4951	TGTCTGTATA	CCGTGCACTT	CTAGCTAGAG	CTTGCGGTAA	TCATGGTCAT
	ACAGACATAT	GGCAGCTGGA	GATCGATCTC	GAACCGCATT	AGTACCAGTA
5001	AGCTGTTTCC	TGTGTGAAAT	TGTTATCCGC	TCACAATTCC	ACACAACATA
	TCGACAAAGG	ACACACTTTA	ACAATAGGCG	AGTGTTAAGG	TGTGTTGTAT
5051	CGAGCCGGAA	GCATAAAGTG	TAAAGCCTGG	GGTGCTTAAT	GAGTGAGCTA
	GCTCGGCCTT	CGTATTTTAC	ATTTGCGACC	CCACGGATTA	CTCACTCGAT
5101	ACTCACATTA	ATTGCGTTGC	GCTCACTGCC	CGCTTTCCAG	TCGGGAAACC
	TGAGTGTAAT	TAACGCAACG	CGAGTGACGG	GCGAAAGGTC	AGCCCTTTGG
5151	TGTCGTGCCA	GCTGCATTAA	TGAATCGGCC	AACGCGCGGG	GAGAGGCGGT
	ACAGCACGGT	CGACGTAATT	ACTTAGCCGG	TTGCGCGCCC	CTCTCCGCCA
5201	TTGCGTATTG	GGCGCTCTTC	CGCTTCCTCG	CTCACTGACT	CGCTGCGCTC
	AACGCATAAC	CCGCGAGAAG	GCGAAGGAGC	GAGTGACTGA	GCGACGCGAG
5251	GGTCGTTCCG	CTGCGGCGAG	CGGTATCAGC	TCACTCAAAG	GCGGTAATAC
	CCAGCAAGCC	GACGCCGCTC	GCCATAGTCG	AGTGAGTTTC	CGCCATTATG
5301	GGTTATCCAC	AGAATCAGGG	GATAACGCAG	GAAAGAACAT	GTGAGCAAAA
	CCAATAGGTG	TCTTAGTCCC	CTATTGCGTC	CTTTCTTGTA	CACTCGTTTT
5351	GGCCAGCAAA	AGGCCAGGAA	CCGTAAAAAG	GCCGCGTTGC	TGGCGTTTTT
	CCGGTCGTTT	TCCGGTCCTT	GGCATTTTTC	CGGCGCAACG	ACCGCAAAAA
5401	CCATAGGCTC	CGCCCCCTTG	ACGAGCATCA	CAAAAATCGA	CGCTCAAGTC
	GGTATCCGAG	GCGGGGGGAC	TGCTCGTAGT	GTTTTTAGCT	GCGAGTTTCA
5451	AGAGGTGGCG	AAACCCGACA	GGACTATAAA	GATACCAGGC	GTTTCCCTCT
	TCTGACCCGC	TTTGGGCTGT	CCTGATATTT	CTATGGTCCG	CAAAGGGGGA
5501	GGAAGCTCCC	TCGTGCGCTC	TCCTGTTCCG	ACCCTGCCGC	TTACCGGATA
	CCTTCGAGGG	AGCACGCGAG	AGGACAAGGC	TGGGACGGCG	AATGGCCTAT
5551	CCTGTCCGCC	TTTCTCCCTT	CGGGAAGCGT	GGCGCTTTCT	CAATGCTCAC
	GGACAGGCCG	AAAGAGGGAA	GCCCTTCGCA	CCGCGAAAGA	GTTACGAGTG
5601	GCTGTAGGTA	TCTCAGTTCC	GTGTAGGTCG	TTCGCTCCAA	GCTGGGCTGT
	CGACATCCAT	AGAGTCAAGC	CACATCCAGC	AAGCGAGGTT	CGACCCGACA

FIG.26E



51/76

5651	GTGCACGAAC	CCCCCGTTCA	GCCCGACCGC	TGCGCCTTAT	CCGGTAACTA
	CACGTGCTTG	GGGGGCAAGT	CGGGCTGGCG	ACGCGGAATA	GGCCATTGAT
5701	TCGTCTTGAG	TCCAACCCGG	TAAGACACGA	CTTATCGCCA	CTGGCAGCAG
	AGCAGAACTC	AGGTTGGGCC	ATTCTGTGCT	GAATAGCGGT	GACCGTCGTC
5751	CCACTGGTAA	CAGGATTAGC	AGAGCGAGGT	ATGTAGGCGG	TGCTACAGAG
	GGTGACCAAT	GTCCTAATCG	TCTCGTCCA	TACATCCGCC	ACGATGTCTC
5801	TTCTTGAAGT	GGTGGCCTAA	CTACGGCTAC	ACTAGAAGGA	CAGTATTTGG
	AAGAACTTCA	CCACCGGATT	GATGCCGATG	TGATCTTCCT	GTCATAAACC
5851	TATCTGCGCT	CTGCTGAAGC	CAGTTACCTT	CGGAAAAAGA	GTTGGTAGCT
	ATAGACGCGA	GACGACTTCG	GTCAATGGAA	GCCTTTTTCT	CAACCATCGA
5901	CTTGATCCGG	CAAACAAACC	ACCGCTGGTA	GCGGTGGTTT	TTTTGTTTGC
	GAACTAGGCC	GTTTGTTTGG	TGGCGACCAT	CGCCACCAAA	AAAACAAACG
5951	AAGCAGCAGA	TTACGCGCAG	AAAAAAGGA	TCTCAAGAAG	ATCCTTTGAT
	TTCGTCGTCT	AATGCGCGTC	TTTTTTCCT	AGAGTTCCTC	TAGGAACTA
6001	CTTTTCTACG	GGGTCTGACG	CTCAGTGGAA	CGAAAACTCA	CGTTAAGGGA
	GAAAAAGATGC	CCCAGACTGC	GAGTCACCTT	GCTTTTGAGT	GCAATTCCCT
6051	TTTTGGTCAT	GAGATTATCA	AAAAGGATCT	TCACCTAGAT	CCTTTTAAAT
	AAAACCAGTA	CTCTAATAGT	TTTTCTAGA	AGTGGATCTA	GGAAAAATTA
6101	TAAAAATGAA	GTTTTAAATC	AATCTAAAGT	ATATATGAGT	AAACTTGGTC
	ATTTTTACTT	CAAAATTTAG	TTAGATTTCA	TATATACTCA	TTTGAACCAG
6151	TGACAGTTAC	CAATGCTTAA	TCAGTGAGGC	ACCTATCTCA	GCGATCTGTC
	ACTGTCATG	GTTACGAATT	AGTCACTCCG	TGGATAGAGT	CGCTAGACAG
6201	TATTTTCGTT	ATCCATAGTT	GCCTGACTCC	CCGTCGTGTA	GATAACTACG
	ATAAAGCAAG	TAGGTATCAA	CGGACTGAGG	GGCAGCACAT	CTATTGATGC
6251	ATACGGGAGG	GCTTACCATC	TGGCCCCAGT	GCTGCAATGA	TACCGCGAGA
	TATGCCCTCC	CGAATGGTAG	ACCGGGGTCA	CGACGTTACT	ATGGCGCTCT
6301	CCCACGCTCA	CCGGCTCCAG	ATTTATCAGC	AATAAACCCAG	CCAGCCGGAA
	GGGTGCGAGT	GGCCGAGGTC	TAAATAGTCG	TTATTTGGTC	GGTGGGCCTT
6351	GGGCCGAGCG	CAGAAGTGGT	CCTGCAACTT	TATCCGCCTC	CATCCAGTCT
	CCCGGCTCGC	GTCTTCACCA	GGACGTTGAA	ATAGGCGGAG	GTAGGTGAGA
6401	ATTAATTGTT	GCCGGGAAGC	TAGAGTAAGT	AGTTCGCCAG	TTAATAGTTT
	TAATTAACAA	CGGCCCTTCG	ATCTCATTCA	TCAAGCGGTC	AATTATCAAA
6451	GCGCAACGTT	GTTGCCATTG	CTACAGGCAT	CGTGGTGTCA	CGCTCGTCGT
	CGCGTTGCAA	CAACGGTAAC	GATGTCCGTA	GCACCACAGT	GCGAGCAGCA
6501	TTGGTATGGC	TTCATTACAG	TCCGTTCCC	AACGATCAAG	GCGAGTTACA
	AACCATACCG	AAGTAAGTCG	AGGCCAAGGG	TTGCTAGTTC	CGCTCAATGT
6551	TGATCCCCCA	TGTTGTGCAA	AAAAGCGGTT	AGCTCCTTCG	GTCCTCCGAT
	ACTAGGGGGT	ACAACACGTT	TTTTCGCCAA	TCGAGGAAGC	CAGGAGGCTA
6601	CGTTGTCAGA	AGTAAGTTGG	CCGCAGTGTT	ATCACTCATG	GTTATGGCAG
	GCAACAGTCT	TCATTCAACC	GGCGTCACAA	TAGTGAGTAC	CAATACCGTC
6651	CACTGCATAA	TTCTCTTACT	GTCATGCCAT	CCGTAAGATG	CTTTTCTGTG
	GTGACGTATT	AAGAGAATGA	CAGTACGGTA	GGCATTCTAC	GAAAAGACAC
6701	ACTGGTGAGT	ACTCAACCAA	GTCATTCTGA	GAATAGTGTA	TGCGGCGACC
	TGACCACTCA	TGAGTTGGTT	CAGTAAGACT	CTTATCACAT	ACGCCGCTGG
6751	GAGTTGCTCT	TGCCCGGCGT	CAATACGGGA	TAATACCGCG	CCACATAGCA
	CTCAACGAGA	ACGGGCCGCA	GTTATGCCCT	ATTATGGCGC	GGTGTATCGT
6801	GAACTTTAAA	AGTGCTCATC	ATTGAAAAAC	GTTCTTCGGG	GCGAAAACTC
	CTTGAAATTT	TCACGAGTAG	TAACCTTTTG	CAAGAAGCCC	CGCTTTTGAG
6851	TCAAGGATCT	TACCGCTGTT	GAGATCCAGT	TCGATGTAAC	CCACTCGTGC
	AGTTCCTAGA	ATGGCGACAA	CTCTAGGTCA	AGCTACATTG	GGTGAGCACG
6901	ACCCAACGTA	TCTTCAGCAT	CTTTTACTTT	CACCAGCGTT	TCTGGGTGAG
	TGGGTTGACT	AGAAGTCGTA	GAAAATGAAA	GTGGTCGCAA	AGACCCACTC
6951	CAAAAACAGG	AAGGCAAAAT	GCCGCAAAAA	AGGGAATAAG	GGCGACACGG
	GTTTTTGTCC	TTCCGTTTTA	CGGCGTTTTT	TCCCTTATTG	CCGCTGTGCC
7001	AAATGTTGAA	TACTCATACT	CTTCCTTTTT	CAATATTATT	GAAGCATTTA
	TTTACAACCT	ATGAGTATGA	GAAGGAAAAA	GTTATAATAA	CTTCGTAAT

FIG.26F

52/76

```
7051 TCAGGGTTAT TGTCTCATGA GCGGATACAT ATTTGAATGT ATTTAGAAAA
      AGTCCCAATA ACAGAGTACT CGCCTATGTA TAAACTTACA TAAATCTTTT
7101 ATAAACAAAT AGGGGTTCCG CGCACATTTT CCCGAAAAGT GCCACCTGAC
      TATTTGTTTA TCCCAAGGC GCGTGTAAG GGGCTTTTCA CGGTGGACTG
7151 GTC
      CAG
```

FIG.26G

53/76

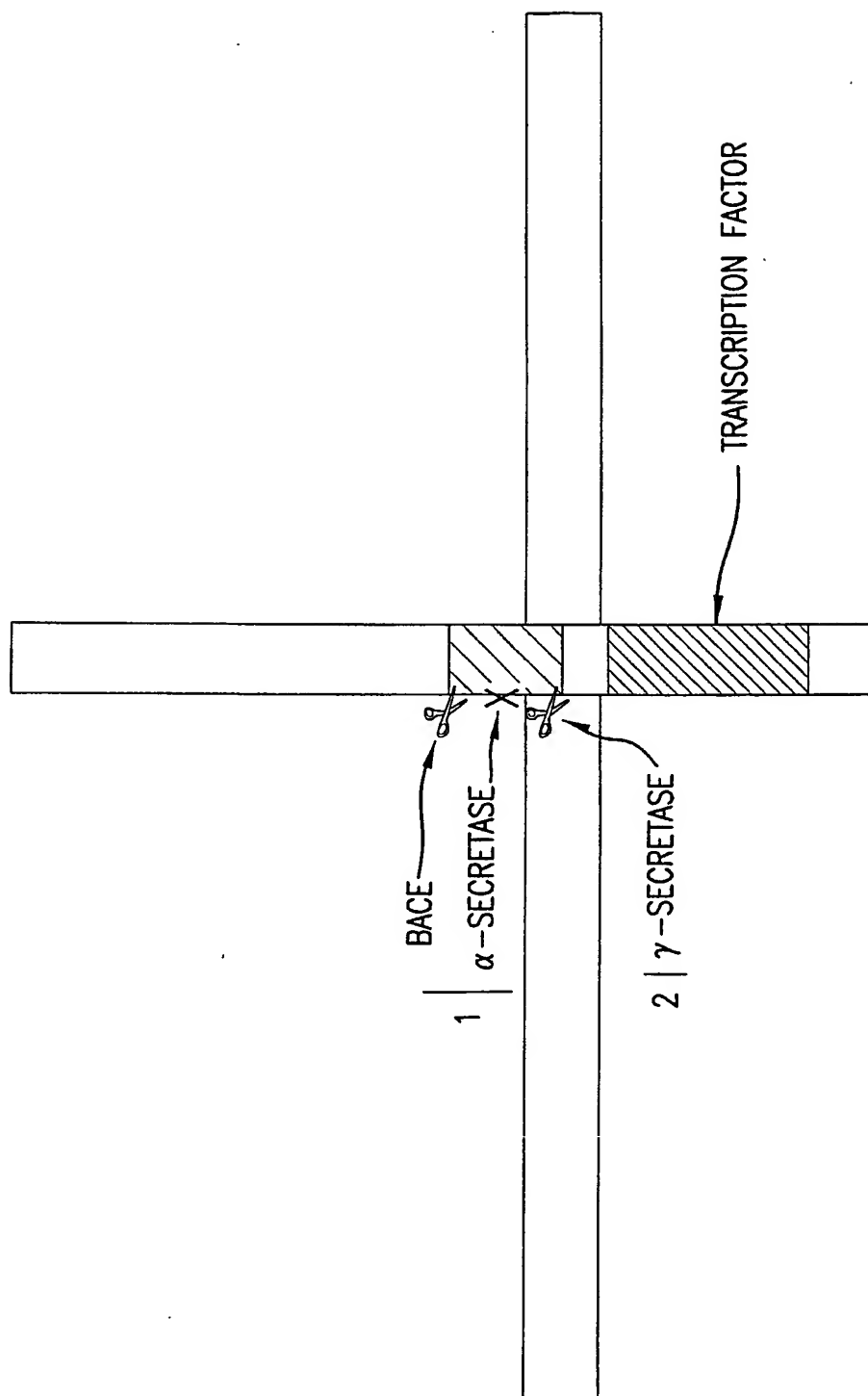


FIG.27A

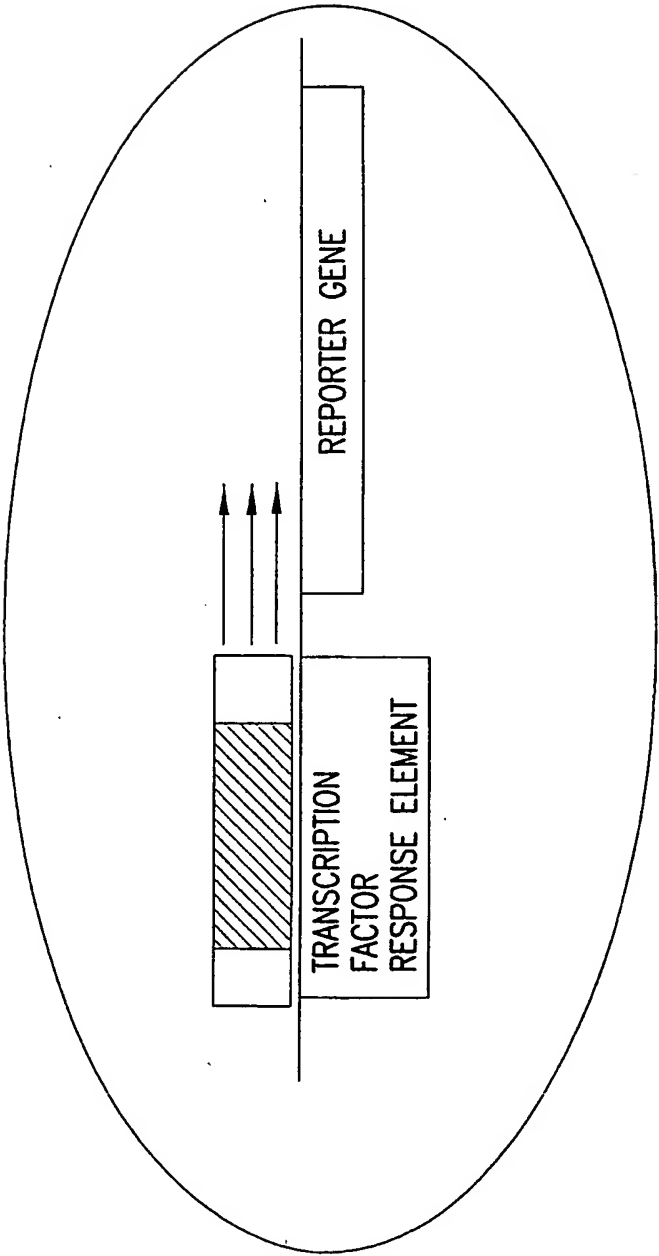


FIG.27B

55/76

DNA sequence of APP(1-651)NFEV, K612V-TATexon1(M1L) APP (664-695)

(SEQ ID NO: 23)

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1 ATGCTGCCCC GTTTGGCACT GCTCCTGCTG GCCGCCTGGA CGGCTCGGGC
51 GCTGGAGGTA CCCACTGATG GTAATGCTGG CCTGCTGGCT GAACCCAGA
101 TTGCCATGTT CTGTGGCAGA CTGAACATGC ACATGAATGT CCAGAATGGG
151 AAGTGGGATT CAGATCCATC AGGGACCAAA ACCTGCATTG ATACCAAGGA
201 AGGCATCCTG CAGTATTGCC AAGAAGCTA CCCTGAACTG CAGATCACCA
251 ATGTGGTAGA AGCCAACCAA CCAGTGACCA TCCAGAACTG GTGCAAGCGG
301 GGCCGCAAGC AGTGCAAGAC CCATCCCCAC TTTGTGATTC CCTACCGCTG
351 CTTAGTTGGT GAGTTTATAA GTGATGCCCT TCTCGTTCCT GACAAGTGCA
401 AATTCTTACA CCAGGAGAGG ATGGATGTTT GCGAAACTCA TCTTCACTGG
451 CACACCGTCG CCAAAGAGAC ATGCAGTGAG AAGAGTACCA ACTTGCATGA
501 CTACGGCATG TTGCTGCCCT GCGGAATTGA CAAGTCCGA GGGGTAGAGT
551 TTGTGTGTTG CCCACTGGCT GAAGAAAGTG ACAATGTGGA TTCTGCTGAT
601 GCGGAGGAGG ATGACTCGGA TGTCTGGTGG GGCGGAGCAG ACACAGACTA
651 TGCAGATGGG AGTGAAGACA AAGTAGTAGA AGTAGCAGAG GAGGAAGAAG
701 TGGCTGAGGT GGAAGAAGAA GAAGCCGATG ATGACGAGGA CGATGAGGAT
751 GGTGATGAGG TAGAGGAAGA GGCTGAGGAA CCCTACGAAG AAGCCACAGA
801 GAGAACCACC AGCATTGCCA CCACCACCAC CACCACCACA GAGTCTGTGG
851 AAGAGGTGGT TCGAGTTCCT ACAACAGCAG CCAGTACCCC TGATGCCGTT
901 GACAAGTATC TCGAGACACC TGGGGATGAG AATGAACATG CCCATTTCCA
951 GAAAGCCAAA GAGAGGCTTG AGGCCAAGCA CCGAGAGAGA ATGTCCCAGG
1001 TCATGAGAGA ATGGGAAGAG GCAGAACGTC AAGCAAAGAA CTTGCCTAAA
1051 GCTGATAAGA AGGCAGTTAT CCAGCATTTT CAGGAGAAAAG TGAATCTTT
1101 GGAACAGGAA GCAGCCAACG AGAGACAGCA GCTGGTGGAG ACACACATGG
1151 CCAGAGTGGA AGCCATGCTC AATGACCGCC GCCGCCTGGC CCTGGAGAAC
1201 TACATCACCG CTCTGCAGGC TGTTCTCCTT CGGCCTCGTC ACGTGTTCAA
1251 TATGCTAAAG AAGTATGTCC GCGCAGAACA GAAGGACAGA CAGCACACCC
1301 TAAAGCATTT CGAGCATGTG CGCATGGTGG ATCCAAGAA AGCCGCTCAG
```

FIG.28A

56/76

1351 ATCCGGTCCC AGGTTATGAC ACACCTCCGT GTGATTTATG AGCGCATGAA  
1401 TCAGTCTCTC TCCCTGCTCT ACAACGTGCC TGCAGTGGCC GAGGAGATTG  
1451 AGGATGAAGT TGATGAGCTG CTTGAGAAAG AGCAAACTA TTCAGATGAC  
1501 GTCTTGGCCA ACATGATTAG TGAACCAAGG ATCAGTTACG GAAACGATGC  
1551 TCTCATGCCA TCTTTGACCG AAACGAAAAC CACCGTGGAG CTCCTTCCCG  
1601 TGAATGGAGA GTTCAGCCTG GACGATCTCC AGCCGTGGCA TTCTTTTGGG  
1651 GCTGACTCTG TGCCAGCCAA CACAGAAAAC GAAGTTGAGC CTGTTGATGC  
1701 CCGCCCTGCT GCCGACCGAG GACTGACCAC TCGACCAGGT TCTGGGTTGA  
1751 CAAATATCAA GACGGAGGAG ATCTCTGAAG TGAACTTTGA AGTGAATTC  
1801 CGACATGACT CAGGATATGA AGTTCATCAT CAAGTATTGG TGTCTTTGC  
1851 AGAAGATGTG GGTTCAAACA AAGGTGCAAT CATTGGACTC ATGGTGGGCG  
1901 GTGTTGTCAT AGCGACAGTG ATCGTCATCA CCTTGGTGAT GCTGAAGAAG  
1951 AAAAAGCTTG GTACCGAGCT CGGATCCACT AGTCCAGTGT GGTGGAATTC  
2001 TGCAGATATC CTGGAGCCAG TAGATCCTAG ACTAGAGCCC TGGGAAGCATC  
2051 CAGGAAGTCA GCCTAAACT GCTTGTACCA ATTGCTATTG TAAAAAGTGT  
2101 TGCTTTCATT GCCAAGTTTG TTTCATGACA AAAGCCTTAG GCATCTCCTA  
2151 TGGCAGGAAG AAGCGGAGAC AGCGACGAAG AGCTCATCAG AACAGTCAGA  
2201 CTCATCAAGC TTCTCTATCA AAGCAGAGGA TATCCAGCAC AGTGGCGGCC  
2251 GCAGACGCCG CTGTCACCCC AGAGGAGCGC CACCTGTCCA AGATGCAGCA  
2301 GAACGGCTAC GAAAATCCAA CCTACAAGTT CTTTGAGCAG ATGCAGAACT  
2351 AG

FIG.28B

57/76

(SEQ ID NO: 24)

Amino acid sequence of APP(1-651)/NFEV, K612V-TATexonI(M1L) APP (664-695):

m l p g l a l l l l a a w t a r a l e v p t d g n a g l l a e p q i a m f c g r l n m h m v n g k w d s d p s g t k t c i d t k e g i l q y c q  
e v y p e l q i t n v e a n q p v t i q n w c k r g r k q c k t h p h f v i p y r c l v g e f i s d a l l v p d k c k f l h q e r m d v c e t h l h  
w h t v a k e t c s e k s t n l h d y g m l p c g i d k f r g v e f v c c p l a e s d n v d s a d a e e d d s d w w g g a d t d y a d g s  
1  
e d k v v e a e e e e v a e v e e e e a d d e d d e d g d e v e e e a e p y e e a t e r t t s i a t t t t t t t s v e e v r v p t t a a s t p d  
a v d k y l e t p g d e n e h a h f q k a k e r l e a k h r e r m s q v m r e w e e a e r q a k n l p k a d k k a v i q h f q e k v e s l e q e  
a a n e r q q l v e t h m a r v e a m l n d r r r l a l e n y i t a l q a v p p r p r h v f n m l k k y v r a e q k d r q h t l k h f e h v r m v d  
p k k a a q i r s q v m t h l r v i y e r m n q s l s l l y n v p a v a e e i q d e v d e l l q k e q n y s d d v l a n m i s e p r i s y g n d a l  
m p s l t e t k t t v e l l p v n g e f s l d d l q p w h s f g a d s v p a n t e n e v e p v d a r p a a d r g l t t r p g s g l t n i k t e e i s e v  
2 3 4 5  
n f e v e f r h d s g y e v h h q l v f f a e d v g s n k g a i g l m v g g v v i a t v i v i t l v m l k k k l g t e l g s t s p w w n s  
6  
a d i l e p v d p r l e p w k h p g s q p k t a c t n c y c k k c c f h c q v c f m t k a l q i s y g r k k r r q r r r a h q n s q t h q a s l s k q  
7 8  
r i s s t v a a a d a a v t p e e r h l s k m q n g y e n p t y k f f e g m q n

FIG. 29

58/76

DNA sequence of APP(1-651)NFEV, K612V-GAL4VP16(delMet) APP (664-695)

(SEQ ID NO: 25)

1 ATGCTGCCCCG GTTTGGCACT GCTCCTGCTG GCCGCCTGGA CGGCTCGGGC  
51 GCTGGAGGTA CCCACTGATG GTAATGCTGG CCTGCTGGCT GAACCCAGAG  
101 TTGCCATGTT CTGTGGCAGA CTGAACATGC ACATGAATGT CCAGAATGGG  
151 AAGTGGGATT CAGATCCATC AGGGACCAAA ACCTGCATTG ATACCAAGGA  
201 AGGCATCCTG CAGTATTGCC AAGAAGTCTA CCCTGAACTG CAGATCACCA  
251 ATGTGGTAGA AGCCAACCAA CCAGTGACCA TCCAGAACTG GTGCAAGCGG  
301 GGCCGCAAGC AGTGCAAGAC CCATCCCCAC TTTGTGATTC CCTACCGCTG  
351 CTTAGTTGGT GAGTTTATAA GTGATGCCCT TCTCGTTCCT GACAAGTGCA  
401 AATTCTTACA CCAGGAGAGG ATGGATGTTT GCGAAACTCA TCTTCACTGG  
451 CACACGTCG CCAAAGAGAC ATGCAGTGAG AAGAGTACCA ACTTGATGA  
501 CTACGGCATG TTGCTGCCCT GCGGAATTGA CAAGTCCGA GGGGTAGAGT  
551 TTGTGTGTTG CCCACTGGCT GAAGAAAGTG ACAATGTGGA TTCTGCTGAT  
601 GCGGAGGAGG ATGACTCGGA TGTCTGGTGG GGCGGAGCAG ACACAGACTA  
651 TGCAGATGGG AGTGAAGACA AAGTAGTAGA AGTAGCAGAG GAGGAAGAAG  
701 TGGCTGAGGT GGAAGAAGAA GAAGCCGATG ATGACGAGGA CGATGAGGAT  
751 GGTGATGAGG TAGAGGAAGA GGCTGAGGAA CCCTACGAAG AAGCCACAGA  
801 GAGAACCACC AGCATTGCCA CCACCACCAC CACCACCACA GAGTCTGTGG  
851 AAGAGGTGGT TCGAGTTCCT ACAACAGCAG CCAGTACCCC TGATGCCGTT  
901 GACAAGTATC TCGAGACACC TGGGGATGAG AATGAACATG CCCATTTCCA  
951 GAAAGCCAAA GAGAGGCTTG AGGCCAAGCA CCGAGAGAGA ATGTCCCAGG  
1001 TCATGAGAGA ATGGGAAGAG GCAGAACGTC AAGCAAAGAA CTTGCCTAAA  
1051 GCTGATAAGA AGGCAGTTAT CCAGCATTTT CAGGAGAAAG TGGAATCTTT  
1101 GGAACAGGAA GCAGCCAACG AGAGACAGCA GCTGGTGGAG ACACACATGG  
1151 CCAGAGTGGG AGCCATGCTC AATGACCGCC GCCGCCTGGC CCTGGAGAAC  
1201 TACATCACCG CTCTGCAGGC TGTTCCTCCT CGGCCTCGTC ACGTGTTCAA

FIG.30A



59/76

1251 TATGCTAAAG AAGTATGTCC GCGCAGAACA GAAGGACAGA CAGCACACCC  
1301 TAAAGCATTT CGAGCATGTG CGCATGGTGG ATCCCAAGAA AGCCGCTCAG  
1351 ATCCGGTCCC AGGTTATGAC ACACCTCCGT GTGATTTATG AGCGCATGAA  
1401 TCAGTCTCTC TCCCTGCTCT ACAACGTGCC TGCAGTGGCC GAGGAGATTC  
1451 AGGATGAAGT TGATGAGCTG CTTCAGAAAG AGCAAACTA TTCAGATGAC  
1501 GTCTTGCCCA ACATGATTAG TGAACCAAGG ATCAGTTACG GAAACGATGC  
1551 TCTCATGCCA TCTTTGACCG AAACGAAAAC CACCGTGGAG CTCCTTCCCG  
1601 TGAATGGAGA GTTCAGCCTG GACGATCTCC AGCCGTGGCA TTCTTTTGGG  
1651 GCTGACTCTG TGCCAGCCAA CACAGAAAAC GAAGTTGAGC CTGTTGATGC  
1701 CCGCCCTGCT GCCGACCGAG GACTGACCAC TCGACCAGGT TCTGGGTGTA  
1751 CAAATATCAA GACGGAGGAG ATCTCTGAAG TGAACTTTGA AGTGAATTC  
1801 CGACATGACT CAGGATATGA AGTTCATCAT CAAGTATTGG TGTCTTTGC  
1851 AGAAGATGTG GGTTCAAACA AAGGTGCAAT CATTGGACTC ATGGTGGGCG  
1901 GTGTTGTCAT AGCGACAGTG ATCGTCATCA CCTTGGTGAT GCTGAAGAAG  
1951 AAAAAGCTTG GTACCGAGCT CGGATCCACT AGTCCAGTGT GGTGGAATTC  
2001 TGCAGATATC AAGCTACTGT CTTCTATCGA ACAAGCATGC GATATTTGCC  
2051 GACTTAAAAA GCTCAAGTGC TCAAAGAAA AACC GAAGTG CGCCAAGTGT  
2101 CTGAAGAACA ACTGGGAGTG TCGCTACTCT CCAAAACCA AAAGGTCTCC  
2151 GCTGACTAGG GCACATCTGA CAGAAGTGA ATCAAGGCTA GAAAGACTGG  
2201 AACAGCTATT TCTACTGATT TTTCTCGAG AAGACCTTGA CATGATTTTG  
2251 AAAATGGATT CTTTACAGGA TATAAAAGCA TTGTAAACAG GATTATTTGT  
2301 ACAAGATAAT GTGAATAAAG ATGCCGTCAC AGATAGATTG GCTTCAGTGG  
2351 AGACTGATAT GCCTCTAACA TTGAGACAGC ATAGAATAAG TGCGACATCA  
2401 TCATCGGAAG AGAGTAGTAA CAAAGGTCAA AGACAGTTGA CTGTATCGGG  
2451 AATTCCCGGG GATCTGGCCC CCCCACCGA TGTACGCTG GGGGACGAGC  
2501 TCCAATTAGA CGGCGAGGAC GTGGCGATGG CGCATGCCA CGCGCTAGAC  
2551 GATTTGATC TGGACATGTT GGGGGACGGG GATTCCCCGG GTCCGGGATT

FIG.30B

60/76

2601 TACCCCCAC GACTCCGCCC CCTACGGCGC TCTGGATATG GCCGACTTCG  
2651 AGTTTGAGCA GATGTTTACC GATGCCCTTG GAATTGACGA GTACGGTGGG  
2701 GATATCCAGC ACAGTGGCGG CCGCGACGCC GCTGTCACCC CAGAGGAGCG  
2751 CCACCTGTCC AAGATGCAGC AGAACGGCTA CGAAAATCCA ACCTACAAGT  
2801 TCTTTGAGCA GATGCAGAAC TAG

**FIG.30C**

61/76

(SEQ ID NO: 26)

Amino acid sequence of APP(1-651)NFEV, K612V, GAL4-VP16(delM1) APP (664-695)

m l p g l a l l l l a a w t a r a l e v p t d g n a g l l a e p q i a m f c g r l n m h m v n g k w d s d p s g t k t c i d t k e g i l q y c q  
e v y p e l q i t n v e a n q p v t i q n w c k r g r k q c k t h p h f v i p y r c l v g e f i s d a l l v p d k c k f l h q e r m d v c e t h l h  
w h t v a k e t c s e k s t n l h d y g m l l p c g i d k f r g v e f v c c p l a e s d n v d s a d a e e d d s d v w g g a d t d y a d g s  
1  
e d k v v e a e e e e v a e v e e e e a d d d e d d e d g d e v e e e a e p y e e a t e r t t s i a t t t t t t t s e v e e v r v p t t a a s t p d  
a v d k y l e t p g d e n e h a h f q k a k e r l e a k h r e r m s q v m r e w e e a e r q a k n l p k a d k k a v i q h f q e k v e s l e q e  
a a n e r q q l v e t h m a r v e a m l n d r r r l a l e n y i t a l q a v p p r p r h v f n m l k k y v r a e q k d r q h t l k h f e h v r m v d  
p k k a a q i r s q v m t h l r v i y e r m n q s l s l l y n v p a v a e e i q d e v d e l l q k e q n y s d d v l a n m i s e p r i s y g n d a l  
m p s l t e t k t t v e l l p v n g e f s l d d l q p w h s f g a d s v p a n t e n e v e p v d a r p a a d r g l t t r p g s g l t n i k t e e i s e v  
2 3 4 5  
n f e v e f r h d s g y e v h h q v l v f f a e d v g s n k g a i g l m v g g v i a t v i v i t l v m l k k k k l g t e l g t s p w m s  
a d i k l l s s i e g a c d i c r l k k l k c s k e k p k c a k c l k n w e c r y s p k t k r s p l t r a h l t e v e s r l e r l e q l f l l i f p r e d l d  
6  
m i l k m d s l q d i k a l l t g l f v q d n v n k d a v t d r l a s v e t d m p l t l r q h r i s a t s s e e s s n k q q r q l t v s q i p g d l a p p  
t d v s l q d e l h l d q e d v a m a h a d a l d d f d l d m l g d q d s p g q f t p h d s a p y g a l d m a d f e f e q m f t d a l g i d e y  
7 8  
g g d i c h s g a a d a a v t p e e r h l s k m q n g y e n p t y k f f e a m n

FIG. 31

62/76

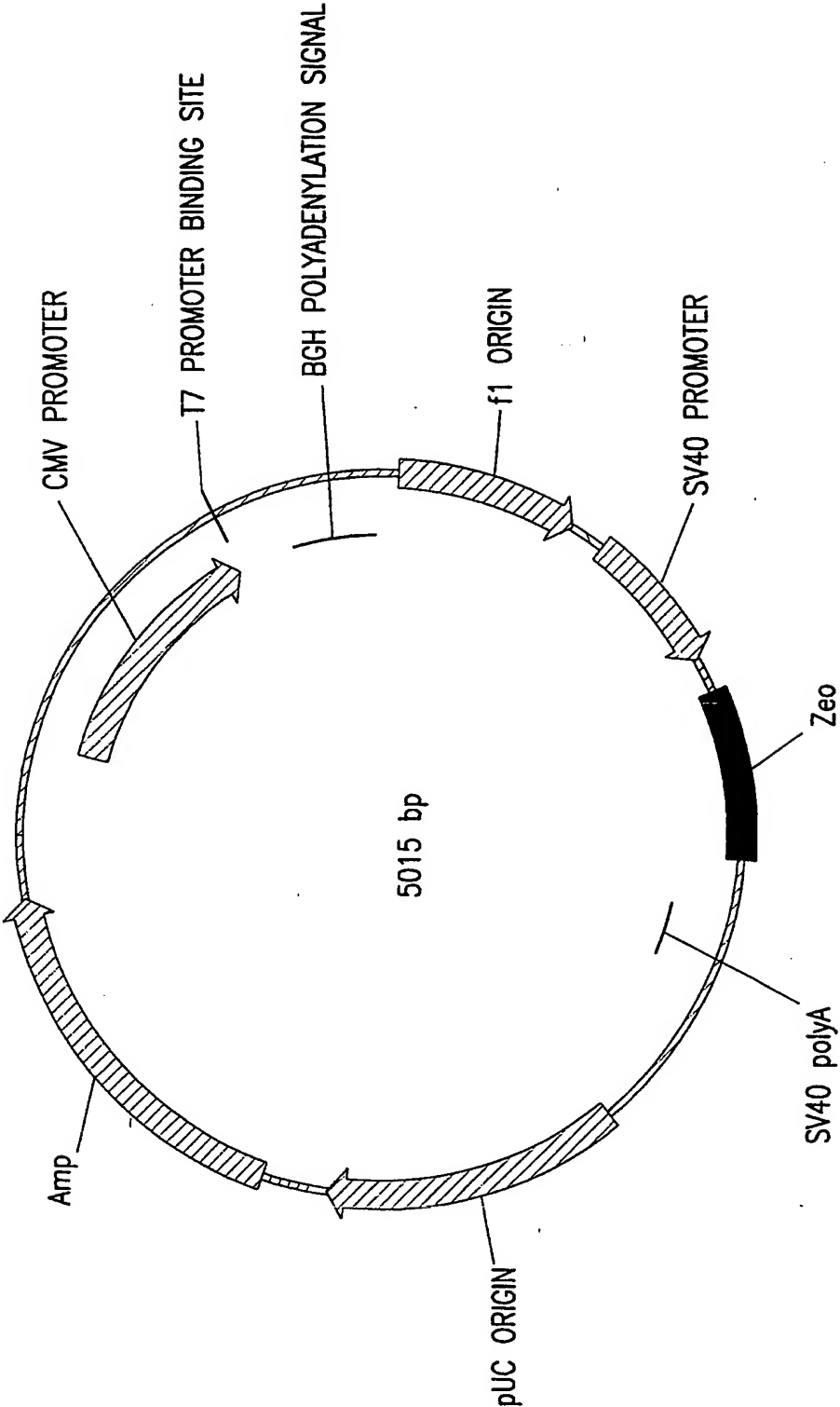


FIG.32A

63/76

(SEQ ID NO: 27 AND 28)

```
1 GACGGATCGG GAGATCTCCC GATCCCCTAT GGTGCGACTCT CAGTACAATC
  CTGCTAGACC CTCTAGAGGG CTAGGGGATA CCAGCTGAGA GTCATGTTAG
51 TGCTCTGATG CCGCATAGTT AAGCCAGTAT CTGCTCCCTG CTTGTGTGTT
  ACGAGACTAC GGCCTATCAA TTCGGTCATA GACGAGGGAC GAACACACAA
101 GGAGGTCGCT GAGTAGTGCG CGAGCAAAAT TTAAGCTACA ACAAGGCAAG
  CCTCCAGCGA CTCATCACGC GCTCGTTTTA AATTCGATGT TGTTCCGTTC
151 GCTTGACCGA CAATTGCATG AAGAATCTGC TTAGGGTTAG GCGTTTTGCG
  CGAACTGGCT GTTAACGTAC TTCTTAGACG AATCCCAATC CGCAAAACGC
201 CTGCTTCGCG ATGTACGGGC CAGATATACG CGTTGACATT GATTATTGAC
  GACGAAGCGC TACATGCCCG GTCTATATGC GCAACTGTAA CTAATAACTG
251 TAGTTATTAA TAGTAATCAA TTACGGGGTC ATTAGTTCAT AGCCCATATA
  ATCAATAATT ATCATTAGTT AATGCCCCAG TAATCAAGTA TCGGGTATAT
301 TGGAGTTCGG CGTTACATAA CTTACGGTAA ATGGCCCCGCC TGGCTGACCG
  ACCTCAAGGC GCAATGTATT GAATGCCATT TACCGGGCGG ACCGACTGGC
351 CCCAACGACC CCCGCCCATT GACGTCAATA ATGACGTATG TTCCCATAGT
  GGGTTGCTGG GGGCGGGTAA CTGCAGTTAT TACTGCATAC AAGGGTATCA
401 AACGCCAATA GGGACTTTCC ATTGACGTCA ATGGGTGGAC TATTTACGGT
  TTGCGGTTAT CCCTGAAAGG TAACTGCAGT TACCCACCTG ATAAATGCCA
451 AAAGTCCCCA CTTGGCAGTA CATCAAGTGT ATCATATGCC AAGTACGCCC
  TTTGACGGGT GAACCGTCAT GTAGTTCACA TAGTATACGG TTCATGCGGG
501 CCTATTGACG TCAATGACGG TAAATGGCCC GCCTGGCATT ATGCCCAGTA
  GGATAACTGC AGTTACTGCC ATTTACGGG CGGACCGTAA TACGGGTCAT
551 CATGACCTTA TGGGACTTTC CTACTTGGCA GTACATCTAC GTATTAGTCA
  GTACTGGAAT ACCCTGAAAG GATGAACCGT CATGTAGATG CATAATCAGT
601 TCGCTATTAC CATGGTGATG CGGTTTTGGC AGTACATCAA TGGGCGTGGA
  AGCGATAATG GTACCACTAC GCCAAAACCG TCATGTAGTT ACCCGCACCT
651 TAGCGGTTTG ACTCACGGGG ATTTCCAAGT CTCCACCCCA TTGACGTCAA
  ATCGCCAAAC TGAGTGCCCC TAAAGGTTCA GAGGTGGGGT AACTGCAGTT
701 TGGGAGTTTG TTTTGGCACC AAAATCAACG GGACTTTCCA AAATGTCGTA
  ACCCTCAAAC AAAACCGTGG TTTTAGTTGC CCTGAAAGGT TTTACAGCAT
751 ACAACTCCGC CCCATTGACG CAAATGGGCG GTAGGCGTGT ACGGTGGGAG
  TGTTGAGGCG GGGTAACTGC GTTTACCCGC CATCCGCACA TGCCACCCCT
801 GTCTATATAA GCAGAGCTCT CTGGCTAACT AGAGAACCCA CTGCTTACTG
  CAGATATATT CGTCTCGAGA GACCGATTGA TCTCTTGGGT GACGAATGAC
851 GCTTATCGAA ATTAATACGA CTCACTATAG GGAGACCCAA GCTGGCTAGC
  CGAATAGCTT TAATTATGCT GAGTGATATC CCTCTGGGTT CGACCGATCG
901 GTTTAAACTT AAGCTTGGTA CCGAGCTCGG ATCCACTAGT CCAGTGTGGT
  CAAATTTGAA TTCGAACCAT GGCTCGAGCC TAGGTGATCA GGTCACACCA
951 GGAATTCTGC AGATATCCAG CACAGTGGCG GCCGCTCGAG TCTAGAGGGC
  CCTTAAGACG TCTATAGGTC GTGTCACCGC CGGCGAGCTC AGATCTCCCG
1001 CCGTTTAAAC CCGCTGATCA GCCTCGACTG TGCCTTCTAG TTGCCAGCCA
  GGCAAATTTG GGGCACTAGT CGGAGCTGAC ACGGAAGATC AACGGTCGGT
1051 TCTGTTGTTT GCCCTCCCC CGTGCCTTCC TTGACCCTGG AAGGTGCCAC
  AGACAACAAA CGGGGAGGGG GCACGGAAGG AACTGGGACC TTCCACGGTG
1101 TCCCACTGTC CTTTCCTAAT AAAATGAGGA AATTGCATCG CATTGTCTGA
  AGGGTGACAG GAAAGGATTA TTTTACTCCT TTAACGTAGC GTAACAGACT
1151 GTAGGTGTCA TTCTATTCTG GGGGGTGGGG TGGGGCAGGA CAGCAAGGGG
  CATCCACAGT AAGATAAGAC CCCCCACCCC ACCCGTCCT GTCGTTCCCC
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FIG.32B

64/76

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1201 GAGGATTGGG AAGACAATAG CAGGCATGCT GGGGATGCGG TGGGCTCTAT
      CTCCTAACCC TTCTGTTATC GTCCGTACGA CCCCTACGCC ACCCGAGATA
1251 GGCTTCTGAG GCGGAAAGAA CCAGCTGGGG CTCTAGGGGG TATCCCCACG
      CCGAAGACTC CGCCTTTCTT GGTCGACCCC GAGATCCCCC ATAGGGGTGC
1301 CGCCCTGTAG CGGCGCATT AAGCGCGCGG GTGTGGTGGT TACGCGCAGC
      GCGGGACATC GCCGCGTAAT TCGCGCCGCC CACACCACCA ATGCGCGTCG
1351 GTGACCGCTA CACTTGCCAG CGCCCTAGCG CCCGCTCCTT TCGCTTTCTT
      CACTGGCGAT GTGAACGGTC GCGGGATCGC GGGCGAGGAA AGCGAAAGAA
1401 CCCTTCCTTT CTCGCCACGT TCGCCGGCTT TCCCCGTCAA GCTCTAAATC
      GGGGAAGGAAA GAGCGGTGCA AGCGGCCGAA AGGGGCAGTT CGAGATTTAG
1451 GGGGCATCCC TTTAGGGTTC CGATTTAGTG CTTTACGGCA CCTCGACCCC
      CCCCGTAGGG AAATCCCAAG GCTAAATCAC GAAATGCCGT GGAGCTGGGG
1501 AAAAACTTG ATTAGGGTGA TGGTTCACGT AGTGGGCCAT CGCCCTGATA
      TTTTTGAAC TAATCCCACT ACCAAGTGCA TCACCCGGTA GCGGGACTAT
1551 GACGGTTTT CGCCCTTTGA CGTTGGAGTC CACGTTCTTT AATAGTGGAC
      CTGCCAAAAA GCGGGAAGCT GCAACCTCAG GTGCAAGAAA TTATCACCTG
1601 TCTTGTTCCT AACTGGAACA ACACTCAACC CTATCTCGGT CTATTCTTTT
      AGAACAAGGT TTGACCTTGT TGTGAGTTGG GATAGAGCCA GATAAGAAAA
1651 GATTTATAAG GGATTTTGGG GATTTTCGGC TATTGGTTAA AAAATGAGAA
      CTAAATATTC CCTAAAAACC CTAAAGCCGG ATAACCAATT TTTTACTCGA
1701 GATTTAACAA AAATTTAACG CGAATTAATT CTGTGGAATG TGTGTCAGTT
      CTAAATTGTT TTTAAATTGC GCTTAATTAA GACACCTTAC ACACAGTCAA
1751 AGGGTGTGGA AAGTCCCGAG GCTCCCGAGG CAGGCAGAAG TATGCAAAGC
      TCCACACCTT TTCAGGGGTC CGAGGGGTCC GTCCGTCTTC ATACGTTTCG
1801 ATGCATCTCA ATTAGTCAGC AACCAGGTGT GGAAAGTCCC CAGGCTCCCC
      TACGTAGAGT TAATCAGTCG TTGGTCCACA CCTTTCAGGG GTCCGAGGGG
1851 AGCAGGCAGA AGTATGCAAA GCATGCATCT CAATTAGTCA GCAACCATAG
      TCGTCCGTCT TCATACGTTT CGTACGTAGA GTTAATCAGT CGTTGGTATC
1901 TCCCGCCCTT AACTCCGCC ATCCCGCCCC TAACTCCGCC CAGTTCGCCC
      AGGGCGGGGA TTGAGGCGGG TAGGGCGGGG ATTGAGGCGG GTCAAGGCGG
1951 CATTCTCCGC CCCATGGCTG ACTAATTTTT TTTATTTATG CAGAGGCCGA
      GTAAGAGGCG GGGTACCGAC TGATTAATAA AAATAAATAC GTCTCCGGCT
2001 GGCCGCCTCT GCCTCTGAGC TATTCCAGAA GTAGTGAGGA GGCTTTTTTG
      CCGGCGGAGA CGGAGACTCG ATAAGGTCTT CATCACTCCT CCGAAAAAAC
2051 GAGGCCTAGG CTTTGTGAAA AAGCTCCCGG GAGCTTGTAT ATCCATTTTC
      CTCGGGATCC GAAAACGTTT TTCGAGGGCC CTCGAACATA TAGGTAAAAG
2101 GGATCTGATC AGCACGTGTT GACAATTAAT CATCGGCATA GTATATCGGC
      CCTAGACTAG TCGTGACAAA CTGTTAATTA GTAGCCGTAT CATATAGCCG
2151 ATAGTATAAT ACGACAAGGT GAGGAATAA ACCATGGCCA AGTTGACCAG
      TATCATATTA TGCTGTTCCA CTCCTTGATT TGGTACCGGT TCAACTGGTC
2201 TGCCGTTCCG GTGCTCACC GCGCGACGT CGCCGGAGCG GTCGAGTTCT
      ACGCAAGGC CACGAGTGGC GCGCGCTGCA GCGGCCTCGC CAGCTCAAGA
2251 GGACCGACCG GCTCGGGTTC TCCCGGGACT TCGTGGAGGA CGACTTCGCC
      CCTGGCTGGC CGAGCCCAAG AGGGCCCTGA AGCACCTCCT GCTGAAGCGG
2301 GGTGTGGTCC GGGACGACGT GACCCTGTTC ATCAGCGCGG TCCAGGACCA
      CCACACCAGG CCCTGCTGCA CTGGGACAAG TAGTCGCGCC AGGTCTGGT
2351 GGTGTGGCCG GACAACACCC TGGCCTGGGT GTGGGTGCGC GGCCTGGACG
      CCACCACGGC CTGTTGTGGG ACCGGACCCA CACCACGCG CCGGACCTGC

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FIG.32C

65/76

2401 AGCTGTACGC CGAGTGGTCG GAGGTCGTGT CCACGAACTT CCGGGACGCC  
TCGACATGCG GCTCACCAGC CTCCAGCACA GGTGCTTGAA GGCCCTGCGG  
2451 TCCGGGCCGG CCATGACCGA GATCGGCGAG CAGCCGTGGG GGCGGGAGTT  
AGGCCCGGCC GGTACTGGCT CTAGCCGCTC GTCGGCACCC CCGCCCTCAA  
2501 CGCCCTGCGC GACCCGGCCG GCAACTGCGT GCACTTCGTG GCCGAGGAGC  
GCGGGACGCG CTGGGCCGGC CGTTGACGCA CGTGAAGCAC CGGCTCCTCG  
2551 AGGACTGACA CGTGCTACGA GATTTTCGATT CCACCGCCGC CTTCTATGAA  
TCCTGACTGT GCACGATGCT CTAAAGCTAA GGTGGCGGGC GAAGATACTT  
2601 AGGTTGGGCT TCGGAATCGT TTTCCGGGAC GCCGGCTGGA TGATCCTCCA  
TCCAACCCGA AGCCTTAGCA AAAGGCCCTG CGGCCGACCT ACTAGGAGGT  
2651 GCGCGGGGAT CTCATGCTGG AGTTCTTCGC CCACCCCAAC TTGTTTATTG  
CGCGCCCTA GAGTACGACC TCAAGAAGCG GGTGGGGTTG AACAAATAAC  
2701 CAGCTTATAA TGGTTACAAA TAAAGCAATA GCATCACAAA TTTCACAAAT  
GTCGAATATT ACCAATGTTT ATTTCTGTTT CGTAGTGTTT AAAGTGTTTA  
2751 AAAGCATTTT TTTCACTGCA TTCTAGTTGT GGTGTGTCCA AACTCATCAA  
TTTCGTAAAA AAAGTGACGT AAGATCAACA CCAAACAGGT TTGAGTAGTT  
2801 TGTATCTTAT CATGTCTGTA TACCGTCGAC CTCTAGCTAG AGCTTGCCGT  
ACATAGAATA GTACAGACAT ATGGCAGCTG GAGATCGATC TCGAACCGCA  
2851 AATCATGGTC ATAGCTGTTT CCTGTGTGAA ATTGTTATCC GCTCACAATT  
TTAGTACCAG TATCGACAAA GGACACACTT TAACAATAGG CGAGTGTTAA  
2901 CCACACAACA TACGAGCCGG AAGCATAAAG TGTAAGCCT GGGGTGCCA  
GGTGTGTTGT ATGCTCGGCC TTCGTATTTT ACATTTTCGA CCCACGGAT  
2951 ATGAGTGAGC TAACTCACAT TAATTGCGTT GCGCTCACTG CCCGCTTTCC  
TACTCACTCG ATTGAGTGTA ATTAACGCAA CGCGAGTGAC GGGCGAAAGG  
3001 ATGCGGGAAA CCTGTCGTGC CAGCTGCATT AATGAATCGG CCAACGCGCG  
TCAGCCCTTT GGACAGCACG GTCGACGTAA TTACTTAGCC GGTGCGCGC  
3051 GGGAGAGGCG GTTTGCGTAT TGGGCGCTCT TCCGCTTCCT CGCTCACTGA  
CCCTCTCCGC CAAACGCATA ACCGCGAGA AGGCGAAGGA GCGAGTGACT  
3101 CTCGCTGCGC TCGGTCGTTT GGCTGCGGCG AGCGGTATCA GCTCACTCAA  
GAGCGACGCG AGCCAGCAAG CCGACGCCGC TCGCCATAGT CGAGTGAGTT  
3151 AGGCGGTAAT ACGGTTATCC ACAGAATCAG GGGATAACGC AGGAAAGAAC  
TCCGCCATTA TGCCAATAGG TGTCTTAGTC CCCTATTGCG TCCTTTCTTG  
3201 ATGTGAGCAA AAGGCCAGCA AAAGGCCAGG AACCGTAAAA AGGCCGCGTT  
TACACTCGTT TTCCGGTCGT TTTCCGGTCC TTGGCATTIT TCCGGCGCAA  
3251 GCTGGCGTTT TTCCATAGGC TCCGCCCCC TGACGAGCAT CACAAAAATC  
CGACCGCAAA AAGGTATCCG AGGCGGGGGG ACTGCTCGTA GTGTTTTTAG  
3301 GACGCTCAAG TCAGAGGTGG CGAAACCCGA CAGGACTATA AAGATACCG  
CTGCGAGTTC AGTCTCCACC GCTTTGGGCT GTCCTGATAT TTCTATGGTC  
3351 GCGTTTCCCC CTGGAAGCTC CCTCGTGCGC TCTCTGTTT CGACCCTGCC  
CGCAAAGGGG GACCTTCGAG GGAGCACGCG AGAGGACAAG GCTGGGACGG  
3401 GCTTACCGGA TACCTGTCCG CCTTTCTCCC TTCGGGAAGC GTGGCGCTTT  
CGAATGGCCT ATGGACAGGC GGAAGAGGG AAGCCCTTCG CACCGCGAAA  
3451 CTCAATGCTC ACGCTGTAGG TATCTCAGTT CGGTGTAGGT CGTTGCTCC  
GAGTTACGAG TGCGACATCC ATAGAGTCAA GCCACATCCA GCAAGCGAGG  
3501 AAGCTGGGCT GTGTGCACGA ACCCCCCGTT CAGCCCGACC GCTGCGCCTT  
TTCGACCCGA CACACGTGCT TGGGGGGCAA GTCGGGCTGG CGACGCGGAA  
3551 ATCCGGTAAC TATCGTCTTG AGTCCAACCC GGTAAGACAC GACTTATCGC  
TAGGCCATTG ATAGCAGAAC TCAGGTTGGG CCATTCTGTG CTGAATAGCG

FIG.32D

66/76

```

3601 CACTGGCAGC AGCCACTGGT AACAGGATTA GCAGAGCGAG GTATGTAGGC
      GTGACCGTCG TCGGTGACCA TTGTCCTAAT CGTCTCGCTC CATACATCCG
3651 GGTGCTACAG AGTTCTTGAA GTGGTGGCCT AACTACGGCT AACTAGAAAG
      CCACGATGTC TCAAGAACTT CACCACCGGA TTGATGCCGA TGTGATCTTC
3701 GACAGTATTT GGTATCTGCG CTCTGCTGAA GCCAGTTACC TTCGGAAAAA
      CTGTCATAAA CCATAGACGC GAGACGACTT CCGTCAATGG AAGCCTTTTT
3751 GAGTTGGTAG CTCTTGATCC GGCAACAAA CCACCGCTGG TAGCGGTGGT
      CTCAACCATC GAGAACTAGG CCGTTTGTTC GGTGGCGACC ATCGCCACCA
3801 TTTTTTGTTC GCAAGCAGCA GATTACGCGC AGAAAAAAG GATCTCAAGA
      AAAAAACAAA CGTTCGTCGT CTAATGCGCG TCTTTTTTTC CTAGAGTTCT
3851 AGATCCTTTG ATCTTTTCTA CGGGGTCTGA CGCTCAGTGG AACGAAACT
      TCTAGGAAAC TAGAAAAGAT GCCCCAGACT GCGAGTCACC TTGCTTTTGA
3901 CACGTTAAGG GATTTTGGTC ATGAGATTAT CAAAAAGGAT CTTACCTAG
      GTGCAATTCC CTAACCACAG TACTCTAATA GTTTTTCCTA GAAGTGGATC
3951 ATCCTTTTAA ATTAATAATG AAGTTTTAAA TCAATCTAAA GTATATATGA
      TAGGAAAAAT TAATTTTAC TTCAAAATTT AGTTAGATT CATATATACT
4001 GTAAACTTGG TCTGACAGTT ACCAATGCTT AATCAGTGAG GCACCTATCT
      CATTTGAACC AGACTGTCAA TGGTTACGAA TTAGTCACTC CGTGGATAGA
4051 CAGCGATCTG TCTATTTCTG TCATCCATAG TTGCCTGACT CCCCGTCGTG
      GTCGCTAGAC AGATAAAGCA AGTAGGTATC AACGGACTGA GGGGCAGCAC
4101 TAGATAACTA CGATACGGGA GGGCTTACCA TCTGGCCCCA GTGCTGCAAT
      ATCTATTGAT GCTATGCCCT CCCGAATGGT AGACCGGGGT CACGACGTTA
4151 GATACCGCGA GACCCACGCT CACCGGCTCC AGATTTATCA GCAATAAACC
      CTATGGCGCT CTGGGTGCGA GTGGCCGAGG TCTAAATAGT CGTTATTTGG
4201 AGCCAGCCGG AAGGGCCGAG CGCAGAAGTG GTCCTGCAAC TTTATCCGCC
      TCGGTGCGCC TTCCCGGCTC GCGTCTTCAC CAGGACGTTG AAATAGGCGG
4251 TCCATCCAGT CTATTAATTG TTGCCGGGAA GCTAGAGTAA GTAGTTCGCC
      AGGTAGGTCA GATAATTAAC AACGGCCCTT CGATCTCATT CATCAAGCGG
4301 AGTTAATAGT TTGCGCAACG TTGTTGCCAT TGCTACAGGC ATCGTGGTGT
      TCAATTATCA AACGCGTTGC AACACGGTA ACGATGTCCG TAGCACCACA
4351 CACGCTCGTC GTTTGGTATG GCTTCATTCA GCTCCGGTTC CCAACGATCA
      GTGCGAGCAG CAAACCATAC CGAAGTAAGT CGAGGCCAAG GGTTGCTAGT
4401 AGGCGAGTTA CATGATCCCC CATGTTGTGC AAAAAAGCGG TTAGCTCCTT
      TCCGCTCAAT GTAGTAGGGG GTACAACACG TTTTTTCGCC AATCGAGGAA
4451 CGGTCTCTCC ATCGTTGTCA GAAGTAAGTT GGCCGCAGTG TTATCACTCA
      GCCAGGAGGC TAGCAACAGT CTTCAATCAA CCGGCGTCAC AATAGTGAGT
4501 TGGTTATGGC AGCACTGCAT AATTCTCTTA CTGTCATGCC ATCCGTAAGA
      ACCAATACCG TCGTGACGTA TTAAGAGAAT GACAGTACGG TAGGCATTCT
4551 TGCTTTTCTG TGAAGGTGA GACTCAACC AAGTCATTCT GAGAATAGTG
      ACGAAAAGAC ACTGACCACT CATGAGTTGG TTCAGTAAGA CTCTTATCAC
4601 TATGCGGCGA CCGAGTTGCT CTTGCCCGGC GTCAATACGG GATAATACCG
      ATACGCCGCT GGCTCAACGA GAACGGGCGG CAGTTATGCC CTATTATGGC
4651 CGCCACATAG CAGAACTTTA AAAGTGCTCA TCATTGAAA ACCTTCTTCG
      GCGGTGTATC GTCTTGAAAT TTTCACGAGT AGTAACCTTT TGCAAGAAGC
4701 GGGCGAAAAA TCTCAAGGAT CTTACCGCTG TTGAGATCCA GTTCGATGTA
      CCCGCTTTTG AGAGTTTCTA GAATGGCGAC AACTCTAGGT CAAGCTACAT
4751 ACCCACTCGT GCACCCAAC GATCTTCAGC ATCTTTTACT TTCACCAGCG
      TGGGTGAGCA CGTGGGTGA CTAAGAGTCG TAGAAAATGA AAGTGGTCGC

```

FIG.32E



67/76

```
4801 TTTCTGGGTG AGCAAAAACA GGAAGGCAAA ATGCCGCAAA AAAGGGAATA
      AAAGACCCAC TCGTTTTTGT CCTTCCGTTT TACGGCGTTT TTCCCTTAT
4851 AGGGCGACAC GGAAATGTTG AATACTCATA CTCTTCCTTT TTCAATATTA
      TCCCGCTGTG CCTTTACAAC TTATGAGTAT GAGAAGGAAA AAGTTATAAT
4901 TTGAAGCATT TATCAGGGTT ATTGTCTCAT GAGCGGATAC ATATTTGAAT
      AACTTCGTAA ATAGTCCCAA TAACAGAGTA CTCGCCTATG TATAAACTTA
4951 GTATTTAGAA AAATAAACAA ATAGGGGTTC CGCGCACATT TCCCCGAAAA
      CATAAATCTT TTTATTTGTT TATCCCAAG GCGCGTGTA AGGGGCTTTT
5001 GTGCCACCTG ACGTC
      CACGGTGGAC TGCAG
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FIG.32F

68/76

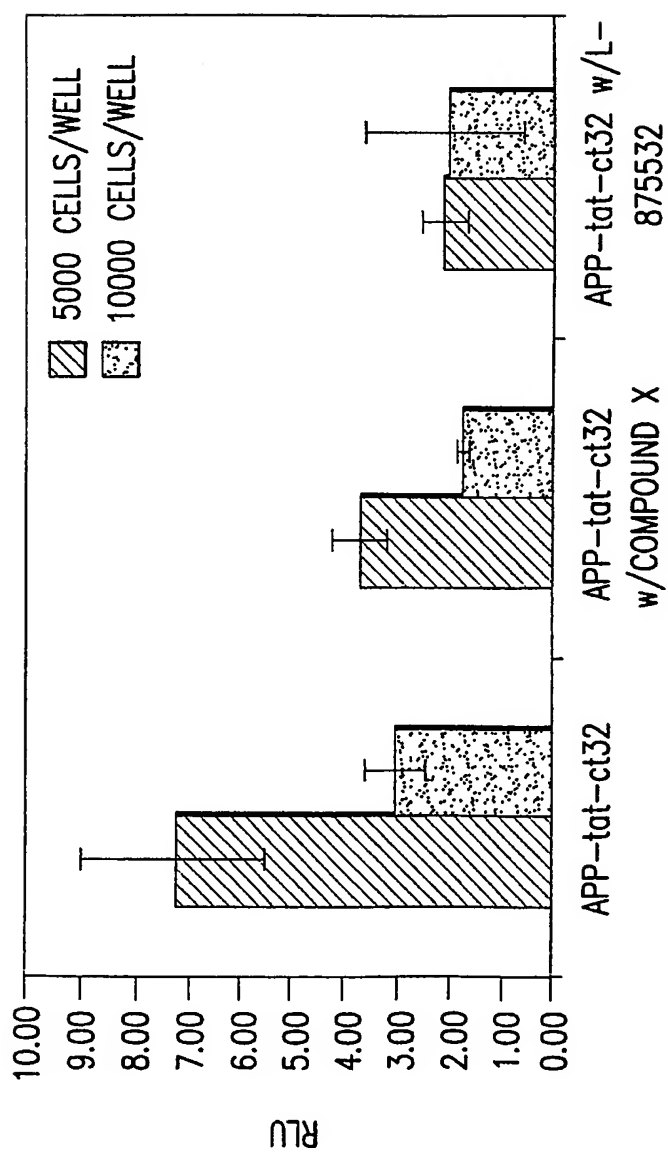


FIG.33

69/76

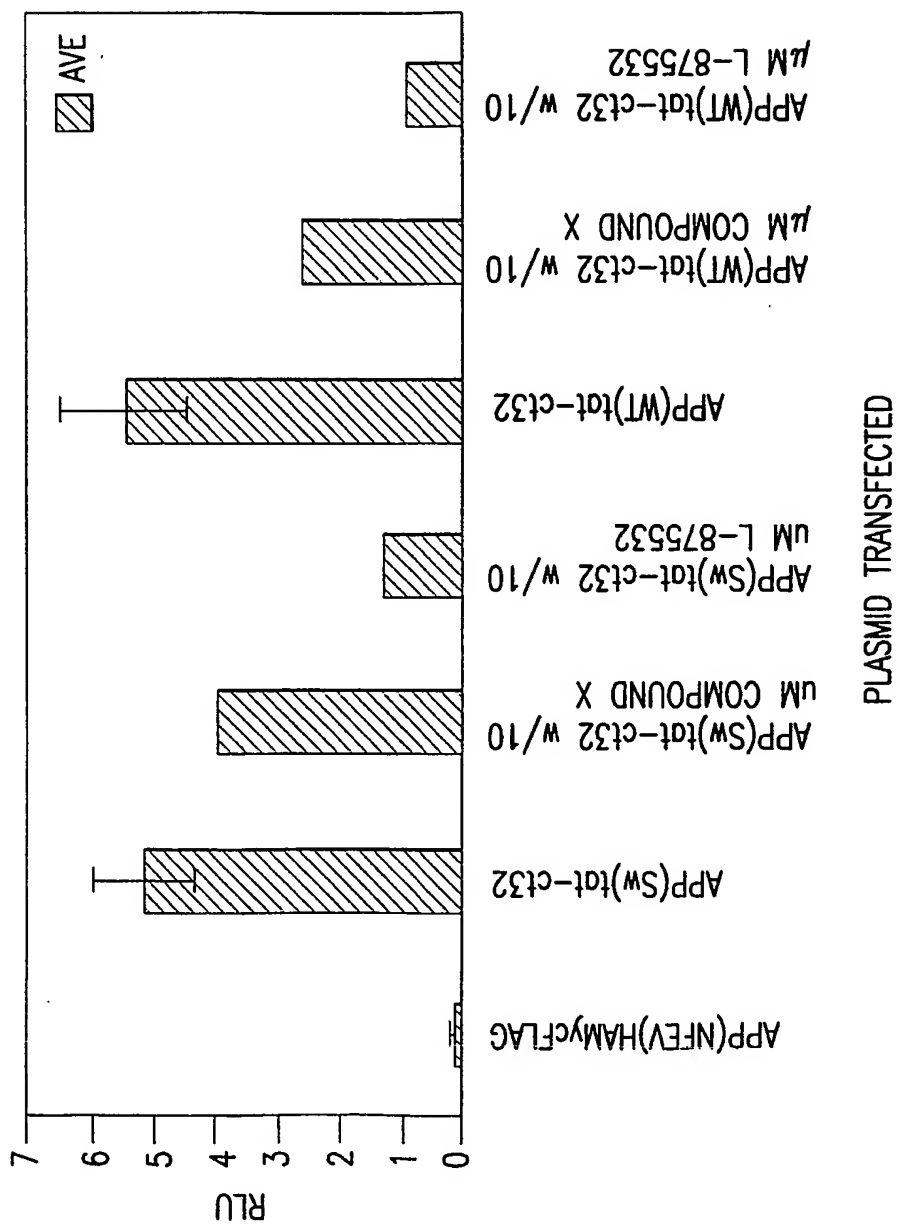


FIG.34

## 70/76

DNA sequence of APP(1-651)NFEV, TATexon1(M1L) APP (664-695)

(SEQ ID NO: 29)

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1 ATGCTGCCCCG GTTTGGCACT GCTCCTGCTG GCCGCCTGGA CGGCTCGGGC
51 GCTGGAGGTA CCCACTGATG GTAATGCTGG CCTGCTGGCT GAACCCAGAG
101 TTGCCATGTT CTGTGGCAGA CTGAACATGC ACATGAATGT CCAGAATGGG
151 AAGTGGGATT CAGATCCATC AGGGACCAAA ACCTGCATTG ATACCAAGGA
201 AGGCATCCTG CAGTATTGCC AAGAAGTCTA CCCTGAACTG CAGATCACCA
251 ATGTGGTAGA AGCCAACCAA CCAGTGACCA TCCAGAACTG GTGCAAGCGG
301 GGCCGCAAGC AGTGCAAGAC CCATCCCCAC TTTGTGATTG CCTACCGCTG
351 CTTAGTTGGT GAGTTTATAA GTGATGCCCT TCTCGTTCCT GACAAGTGCA
401 AATTCTTACA CCAGGAGAGG ATGGATGTTT GCGAAACTCA TCTTCACTGG
451 CACACCGTCG CCAAAGAGAC ATGCAGTGAG AAGAGTACCA ACTTGCATGA
501 CTACGGCATG TTGCTGCCCT GCGGAATTGA CAAGTTCCGA GGGGTAGAGT
551 TTGTGTGTTG CCCACTGGCT GAAGAAAGTG ACAATGTGGA TTCTGCTGAT
601 GCGGAGGAGG ATGACTCGGA TGTCTGGTGG GCGGAGCAG ACACAGACTA
651 TGCAGATGGG AGTGAAGACA AAGTAGTAGA AGTAGCAGAG GAGGAAGAAG
701 TGGCTGAGGT GGAAGAAGAA GAAGCCGATG ATGACGAGGA CGATGAGGAT
751 GGTGATGAGG TAGAGGAAGA GGCTGAGGAA CCCTACGAAG AAGCCACAGA
801 GAGAACCACC AGCATTGCCA CCACCACCAC CACCACCACA GAGTCTGTGG
851 AAGAGGTGGT TCGAGTTCCT ACAACAGCAG CCAGTACCCC TGATGCCGTT
901 GACAAGTATC TCGAGACACC TGGGGATGAG AATGAACATG CCCATTTCCA
951 GAAAGCCAAA GAGAGGCTTG AGGCCAAGCA CCGAGAGAGA ATGTCCCAGG
1001 TCATGAGAGA ATGGGAAGAG GCAGAACGTC AAGCAAAGAA CTTGCCTAAA
1051 GCTGATAAGA AGGCAGTTAT CCAGCATTTT CAGGAGAAAAG TGAATCTTT
1101 GGAACAGGAA GCAGCCAACG AGAGACAGCA GCTGGTGGAG ACACACATGG
1151 CCAGAGTGGA AGCCATGCTC AATGACCGCC GCCGCCTGGC CCTGGAGAAC
1201 TACATACCGG CTCTGCAGGC TGTTCTCCTT CGGCCTCGTC ACGTGTTCAG
1251 TATGCTAAAG AAGTATGTCC GCGCAGAACA GAAGGACAGA CAGCACACCC
1301 TAAAGCATTT CGAGCATGTG CGCATGGTGG ATCCAAGAA AGCCGCTCAG
```

FIG.35A

71/76

1351 ATCCGGTCCC AGGTTATGAC ACACCTCCGT GTGATTTATG AGCGCATGAA  
1401 TCAGTCTCTC TCCCTGCTCT ACAACGTGCC TGCAGTGGCC GAGGAGATTC  
1451 AGGATGAAGT TGATGAGCTG CTTCAGAAAG AGCAAACTA TTCAGATGAC  
1501 GTCTTGGCCA ACATGATTAG TGAACCAAGG ATCAGTTACG GAAACGATGC  
1551 TCTCATGCCA TCTTTGACCG AAACGAAAAC CACCGTGGAG CTCCTTCCCG  
1601 TGAATGGAGA GTTCAGCCTG GACGATCTCC AGCCGTGGCA TTCTTTTGGG  
1651 GCTGACTCTG TGCCAGCCAA CACAGAAAAC GAAGTTGAGC CTGTTGATGC  
1701 CCGCCCTGCT GCCGACCGAG GACTGACCAC TCGACCAGGT TCTGGGTTGA  
1751 CAAATATCAA GACGGAGGAG ATCTCTGAAG TGAACTTTGA AGTGGAATTC  
1801 CGACATGACT CAGGATATGA AGTTCATCAT CAAAAATTGG TGTTCTTTGC  
1851 AGAAGATGTG GGTTCAAACA AAGGTGCAAT CATTGGAATC ATGGTGGGCG  
1901 GTGTTGTCAT AGCGACAGTG ATCGTCATCA CCTTGGTGAT GCTGAAGAAG  
1951 AAAAAGCTTG GTACCGAGCT CGGATCCACT AGTCCAGTGT GGTGGAATTC  
2001 TGCAGATATC CTGGAGCCAG TAGATCCTAG ACTAGAGCCC TGAAGCATC  
2051 CAGGAAGTCA GCCTAAACT GCTTGATCCA ATTGCTATTG TAAAAAGTGT  
2101 TGCTTTCATT GCCAAGTTTG TTTCATGACA AAAGCCTTAG GCATCTCCTA  
2151 TGGCAGGAAG AAGCGGAGAC AGCGACGAAG AGCTCATCAG AACAGTCAGA  
2201 CTCATCAAGC TTCTCTATCA AAGCAGAGGA TATCCAGCAC AGTGGCGGCC  
2251 GCAGACGCCG CTGTCACCCC AGAGGAGCGC CACCTGTCCA AGATGCAGCA  
2301 GAACGGCTAC GAAAATCCAA CCTACAAGTT CTTTGAGCAG ATGCAGAACT  
2351 AG

FIG.35B

72/76

(SEQ ID NO: 30)

Amino acid sequence of APP(1-651)/NFEV, K612V-TATexonI(M1L) APP (664-695)

m l p g l a l l l l a a w t a r a l e v p t d g n a g l l a e p q i a m f c g r l n m h m v n q n g k w d s d p s g t k t c i d t k e g i l q y c q  
e v y p e l q i t n v e a n q p v t i q n w c k r g r k q c k t h p h f v i p y r c l v g e f i s d a l l v p d k c k f l h q e r m d v c e t h l h  
w h t v a k e t c s e k s t n l h d y g m l l p c g i d k f r g v e f v c c p l a e e s d n v d s a d a e e d d s d v w g g a d t d y a d g s  
1  
e d k v v e a e e e e v a e v e e e e a d d e d d e d g d e v e e e a e e p y e e a t e r t t s i a t t t t t t t s v e e v r v p t t a a s t p d  
a v d k y l e t p g d e n e h a h f q k a k e r l e a k h r e r m s q v m r e w e e a e r q a k n l p k a d k k a v i q h f q e k v e s l e q e  
a a n e r q q l v e t h m a r v e a m l n d r r r l a l e n y i t a l q a v p p r p r h v f n m l k k y v r a e q d r q h t l k h f e h v r m v d  
p k k a a q i r s q v m t h l r v i y e r m n q s l s l y n v p a v a e e i q d e v d e l l q e q n y s d d v l a n m i s e p r i s y g n d a l  
m p s l t e t k t t v e l l p v n g e f s l d d l q p w h s f g a d s v p a n t e n e v e p v d a r p a a d r g l t t r p g s g l t n i k t e e i s e v  
2 3 4 5  
n f e v e f r h d s g y e v h h q k l v f f a e d v g s n k g a i g l m v g g v v i a t v i v i t l v m l k k k l g t e l g s t s p w w n s  
6  
a d i l e p v d p r l e p w k h p g s q p k t a c t n c y c k k c f h c q v c f m t k a l g i s y g r k r r r r r a h q n s q t h q a s l s k q  
7 8  
r i s s t v a a a d a a v t p e e r h l s k m a g n g y e n p t y k f f e q m a n

FIG. 36

73/76

DNA sequence of APP(1-651)NFEV, GAL4VP16(delMet) APP (664-695)  
(SEQ ID NO: 31)

```
1 ATGCTGCCCC GTTTGGCACT GCTCCTGCTG GCCGCCTGGA CGGCTCGGGC
51 GCTGGAGGTA CCCACTGATG GTAATGCTGG CCTGCTGGCT GAACCCAGA
101 TTGCCATGTT CTGTGGCAGA CTGAACATGC ACATGAATGT CCAGAATGGG
151 AAGTGGGATT CAGATCCATC AGGGACCAAA ACCTGCATTG ATACCAAGGA
201 AGGCATCCTG CAGTATTGCC AAGAAGTCTA CCCTGAACTG CAGATCACCA
251 ATGTGGTAGA AGCCAACCAA CCAGTGACCA TCCAGAACTG GTGCAAGCGG
301 GGCCGCAAGC AGTGCAAGAC CCATCCCCAC TTTGTGATTC CCTACCGCTG
351 CTTAGTTGGT GAGTTTATAA GTGATGCCCT TCTCGTTCCT GACAAGTGCA
401 AATTCTTACA CCAGGAGAGG ATGGATGTTT GCGAAACTCA TCTTCACTGG
451 CACACCGTCG CCAAAGAGAC ATGCAGTGAG AAGAGTACCA ACTTGCATGA
501 CTACGGCATG TTGCTGCCCT GCGGAATTGA CAAGTCCGA GGGGTAGAGT
551 TTGTGTGTTG CCCACTGGCT GAAGAAAGTG ACAATGTGGA TTCTGCTGAT
601 GCGGAGGAGG ATGACTCGGA TGTCTGGTGG GGCGGAGCAG ACACAGACTA
651 TGCAGATGGG AGTGAAGACA AAGTAGTAGA AGTAGCAGAG GAGGAAGAAG
701 TGGCTGAGGT GGAAGAAGAA GAAGCCGATG ATGACGAGGA CGATGAGGAT
751 GGTGATGAGG TAGAGGAAGA GGCTGAGGAA CCCTACGAAG AAGCCACAGA
801 GAGAACCACC AGCATTGCCA CCACCACCAC CACCACCACA GAGTCTGTGG
851 AAGAGGTGGT TCGAGTTCCT ACAACAGCAG CCAGTACCCC TGATGCCGTT
901 GACAAGTATC TCGAGACACC TGGGGATGAG AATGAACATG CCCATTTCCA
951 GAAAGCCAAA GAGAGGCTTG AGGCCAAGCA CCGAGAGAGA ATGTCCCAGG
1001 TCATGAGAGA ATGGGAAGAG GCAGAACGTC AAGCAAAGAA CTTGCCTAAA
1051 GCTGATAAGA AGGCAGTTAT CCAGCATTTT CAGGAGAAAG TGGAATCTTT
1101 GGAACAGGAA GCAGCCAACG AGAGACAGCA GCTGGTGGAG ACACACATGG
1151 CCAGAGTGGA AGCCATGCTC AATGACCGCC GCCGCCTGGC CCTGGAGAAC
1201 TACATCACCG CTCTGCAGGC TGTTCCTCCT CGGCCTCGTC ACGTGTTCAA
```

FIG.37A

74/76

1251 TATGCTAAAG AAGTATGTCC GCGCAGAACA GAAGGACAGA CAGCACACCC  
1301 TAAAGCATTT CGAGCATGTG CGCATGGTGG ATCCCAAGAA AGCCGCTCAG  
1351 ATCCGGTCCC AGGTTATGAC ACACCTCCGT GTGATTTATG AGCGCATGAA  
1401 TCAGTCTCTC TCCCTGCTCT ACAACGTGCC TGCAGTGGCC GAGGAGATTC  
1451 AGGATGAAGT TGATGAGCTG CTTCAGAAAG AGCAAACTA TTCAGATGAC  
1501 GTCTTGCCA ACATGATTAG TGAACCAAGG ATCAGTTACG GAAACGATGC  
1551 TCTCATGCCA TCTTTGACCG AAACGAAAAC CACCGTGGAG CTCCTTCCCG  
1601 TGAATGGAGA GTTCAGCCTG GACGATCTCC AGCCGTGGCA TTCTTTTGGG  
1651 GCTGACTCTG TGCCAGCCAA CACAGAAAAC GAAGTTGAGC CTGTTGATGC  
1701 CCGCCCTGCT GCCGACCGAG GACTGACCAC TCGACCAGGT TCTGGGTTGA  
1751 CAAATATCAA GACGGAGGAG ATCTCTGAAG TGAACTTTGA AGTGGAATTC  
1801 CGACATGACT CAGGATATGA AGTTCATCAT CAAAAATTGG TGTTCTTTCG  
1851 AGAAGATGTG GGTTCAAACA AAGGTGCAAT CATTGGACTC ATGGTGGGCG  
1901 GTGTTGTCAT AGCGACAGTG ATCGTCATCA CCTTGGTGAT GCTGAAGAAG  
1951 AAAAAGCTTG GTACCGAGCT CGGATCCACT AGTCCAGTGT GGTGGAATTC  
2001 TGCAGATATC AAGCTACTGT CTTCTATCGA ACAAGCATGC GATATTTGCC  
2051 GACTTAAAAA GCTCAAGTGC TCCAAAGAAA AACC GAAGTG CGCCAAGTGT  
2101 CTGAAGAACA ACTGGGAGTG TCGCTACTCT CCCAAAACCA AAAGGTCTCC  
2151 GCTGACTAGG GCACATCTGA CAGAAGTGGA ATCAAGGCTA GAAAGACTGG  
2201 AACAGCTATT TCTACTGATT TTTCTCGAG AAGACCTTGA CATGATTTTG  
2251 AAAATGGATT CTTTACAGGA TATAAAAGCA TTGTTAACAG GATTATTTGT  
2301 ACAAGATAAT GTGAATAAAG ATGCCGTCAC AGATAGATTG GCTTCAGTGG  
2351 AGACTGATAT GCCTCTAACA TTGAGACAGC ATAGAATAAG TGCGACATCA  
2401 TCATCGGAAG AGAGTAGTAA CAAAGGTCAA AGACAGTTGA CTGTATCGGG  
2451 AATTCCCGGG GATCTGGCCC CCCC GACCGA TGTCAGCCTG GGGGACGAGC  
2501 TCCACTTAGA CGGCGAGGAC GTGGCGATGG CGCATGCCGA CGCGCTAGAC  
2551 GATTTGATC TGGACATGTT GGGGGACGGG GATTCCCCGG GTCCGGGATT

FIG.37B



75/76

2601 TACCCCCAC GACTCCGCCC CCTACGGCGC TCTGGATATG GCCGACTTCG  
2651 AGTTTGAGCA GATGTTTACC GATGCCCTTG GAATTGACGA GTACGGTGGG  
2701 GATATCCAGC ACAGTGGCGG CCGCGACGCC GCTGTCACCC CAGAGGAGCG  
2751 CCACCTGTCC AAGATGCAGC AGAACGGCTA CGAAAATCCA ACCTACAAGT  
2801 TCTTTGAGCA GATGCAGAAC TAG

FIG.37C

76/76

(SEQ ID NO: 32)

Amino acid sequence of APP(1-651)NFEV, GAL4-VP16(delM1) APP (664-695)

mlpglallllaaawtaralevptdgnagllaepqiamfcgrlnmhmnvqngkwdsdpsgtktcidtkegilqycq  
 evypelqitnvveanqpvtiqnwckrgrkqckthphfviyrclygefisdallvpdkckflhqermdvcethlh  
 whtvaketcsekstnlhdygmllpcgidkfrgvefvccplaeesdnvdsadaeeddsdvwggadtdyadgs  
 edkvvevaeeeeveveeeeadddeddedgdeveeeaeepyeeaterttsiatTTTTTTesveevrvpttaastpd  
 avdkyletpgdenehahfqkakerleakhrermsqvmreweeaerqaknlpkadkkaviqhfqekvesleqe  
 aanerqqlvethmarveamlnrrrrlalenyitalqavpprrhvfnmkkkyvraeqkdrqhtlkhfehvrmvd  
 pkkaaqrsvqmthlrviyermnqslsllynvpavaeeiqdevdellqkeqnysddvlnmisseprisynadl  
 mpsltetkttvellpvngefslddlqpwhsfgadsvpantenevepvdarpaadrglttrpgsgltnikteeisev  
 nfevefrhdsgyevhhqklvffaedvgsnkgaiiglmvggyviatvivitlmlkkkkltelgstspwms  
 adtkllssiegacdicrlkklkcskekpkcakclknwecryspktrsp1trahltevesrlerleqlfllifpredld  
 milkmdslqdikalltg1fvqdnvkdavtdrlasvetdmp1tlrqhrisatssseessnkqqrqltvsgipgd1app  
 tdvslqdelhldgedvamahadalddfdldmlqdgadspqgftphdsapyqaldmadfefeqmftdalqidey  
 ggdiqhsгаааааavtpeerhlskmaqngyenptykffeqmqn

FIG.38

## SEQUENCE LISTING

<110> Merck & Co., Inc.  
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<120> ASSAYS TO MONITOR AMYLOID PRECURSOR  
PROTEIN PROCESSING

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<150> 60/360,274

<151> 2002-02-27

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cagatcacca atgtggtaga agccaaccaa ccagtgaaca tccagaactg gtgcaagcgg 300
ggccgcaagc agtgcaagac ccatcccccac tttgtgattc cctaccgctg cttagtgggt 360
gagtttataa gtgatgccct tctcgttcct gacaagtgca aattcttaca ccaggagagg 420
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ggggtagagt ttgtgtgttg cccactggct gaagaaagtg acaatgtgga ttctgctgat 600
gcggaggagg atgactcgga tgtctggttg ggccggagcag acacagacta tgcagatggg 660
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&lt;210&gt; 2

&lt;211&gt; 783

&lt;212&gt; PRT

&lt;213&gt; fusion protein - human

&lt;400&gt; 2

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Gln Ile Ala Met Phe Cys Gly Arg Leu Asn Met His Met Asn Val Gln
      35           40           45
Asn Gly Lys Trp Asp Ser Asp Pro Ser Gly Thr Lys Thr Cys Ile Asp
      50           55           60
Thr Lys Glu Gly Ile Leu Gln Tyr Cys Gln Glu Val Tyr Pro Glu Leu
      65           70           75           80
Gln Ile Thr Asn Val Val Glu Ala Asn Gln Pro Val Thr Ile Gln Asn
      85           90           95
Trp Cys Lys Arg Gly Arg Lys Gln Cys Lys Thr His Pro His Phe Val
      100          105          110
Ile Pro Tyr Arg Cys Leu Val Gly Glu Phe Ile Ser Asp Ala Leu Leu
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Val Pro Asp Lys Cys Lys Phe Leu His Gln Glu Arg Met Asp Val Cys
      130          135          140
Glu Thr His Leu His Trp His Thr Val Ala Lys Glu Thr Cys Ser Glu
      145          150          155          160
Lys Ser Thr Asn Leu His Asp Tyr Gly Met Leu Leu Pro Cys Gly Ile
      165          170          175
Asp Lys Phe Arg Gly Val Glu Phe Val Cys Cys Pro Leu Ala Glu Glu
      180          185          190
Ser Asp Asn Val Asp Ser Ala Asp Ala Glu Glu Asp Asp Ser Asp Val
      195          200          205
Trp Trp Gly Gly Ala Asp Thr Asp Tyr Ala Asp Gly Ser Glu Asp Lys
      210          215          220
Val Val Glu Val Ala Glu Glu Glu Val Ala Glu Val Glu Glu Glu
      225          230          235          240
Glu Ala Asp Asp Asp Glu Asp Asp Glu Asp Gly Asp Glu Val Glu Glu
      245          250          255
Glu Ala Glu Glu Pro Tyr Glu Glu Ala Thr Glu Arg Thr Thr Ser Ile
      260          265          270
Ala Thr Thr Thr Thr Thr Thr Thr Glu Ser Val Glu Glu Val Val Arg
      275          280          285
Val Pro Thr Thr Ala Ala Ser Thr Pro Asp Ala Val Asp Lys Tyr Leu
      290          295          300
Glu Thr Pro Gly Asp Glu Asn Glu His Ala His Phe Gln Lys Ala Lys
      305          310          315          320
Glu Arg Leu Glu Ala Lys His Arg Glu Arg Met Ser Gln Val Met Arg
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Glu Trp Glu Glu Ala Glu Arg Gln Ala Lys Asn Leu Pro Lys Ala Asp
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    385          390          395          400
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    405          410          415
Asn Met Leu Lys Lys Tyr Val Arg Ala Glu Gln Lys Asp Arg Gln His
    420          425          430
Thr Leu Lys His Phe Glu His Val Arg Met Val Asp Pro Lys Lys Ala
    435          440          445
Ala Gln Ile Arg Ser Gln Val Met Thr His Leu Arg Val Ile Tyr Glu
    450          455          460
Arg Met Asn Gln Ser Leu Ser Leu Leu Tyr Asn Val Pro Ala Val Ala
    465          470          475          480
Glu Glu Ile Gln Asp Glu Val Asp Glu Leu Leu Gln Lys Glu Gln Asn
    485          490          495
Tyr Ser Asp Asp Val Leu Ala Asn Met Ile Ser Glu Pro Arg Ile Ser
    500          505          510
Tyr Gly Asn Asp Ala Leu Met Pro Ser Leu Thr Glu Thr Lys Thr Thr
    515          520          525
Val Glu Leu Leu Pro Val Asn Gly Glu Phe Ser Leu Asp Asp Leu Gln
    530          535          540
Pro Trp His Ser Phe Gly Ala Asp Ser Val Pro Ala Asn Thr Glu Asn
    545          550          555          560
Glu Val Glu Pro Val Asp Ala Arg Pro Ala Ala Asp Arg Gly Leu Thr
    565          570          575
Thr Arg Pro Gly Ser Gly Leu Thr Asn Ile Lys Thr Glu Glu Ile Ser
    580          585          590
Glu Val Asn Leu Asp Ala Glu Phe Arg His Asp Ser Gly Tyr Glu Val
    595          600          605
His His Gln Val Leu Val Phe Phe Ala Glu Asp Val Gly Ser Asn Lys
    610          615          620
Gly Ala Ile Ile Gly Leu Met Val Gly Gly Val Val Ile Ala Thr Val
    625          630          635          640
Ile Val Ile Thr Leu Val Met Leu Lys Lys Lys Leu Gly Thr Glu
    645          650          655
Leu Gly Ser Thr Ser Pro Val Trp Trp Asn Ser Ala Asp Ile Leu Glu
    660          665          670
Pro Val Asp Pro Arg Leu Glu Pro Trp Lys His Pro Gly Ser Gln Pro
    675          680          685
Lys Thr Ala Cys Thr Asn Cys Tyr Cys Lys Lys Cys Cys Phe His Cys
    690          695          700
Gln Val Cys Phe Met Thr Lys Ala Leu Gly Ile Ser Tyr Gly Arg Lys
    705          710          715          720
Lys Arg Arg Gln Arg Arg Ala His Gln Asn Ser Gln Thr His Gln
    725          730          735
Ala Ser Leu Ser Lys Gln Arg Ile Ser Ser Thr Val Ala Ala Ala Asp
    740          745          750
Ala Ala Val Thr Pro Glu Glu Arg His Leu Ser Lys Met Gln Gln Asn
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<210> 3
<211> 2352
<212> DNA
<213> fusion protein - human

<400> 3

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WO 03/072041

PCT/US03 PCT/US03/05458

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atgcagaact ag                                     2352

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&lt;210&gt; 4

&lt;211&gt; 783

&lt;212&gt; PRT

&lt;213&gt; fusion protein - human

&lt;400&gt; 4

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Ala Leu Glu Val Pro Thr Asp Gly Asn Ala Gly Leu Leu Ala Glu Pro
 20          25          30
Gln Ile Ala Met Phe Cys Gly Arg Leu Asn Met His Met Asn Val Gln
 35          40          45
Asn Gly Lys Trp Asp Ser Asp Pro Ser Gly Thr Lys Thr Cys Ile Asp
 50          55          60
Thr Lys Glu Gly Ile Leu Gln Tyr Cys Gln Glu Val Tyr Pro Glu Leu
 65          70          75          80
Gln Ile Thr Asn Val Val Glu Ala Asn Gln Pro Val Thr Ile Gln Asn
 85          90          95
Trp Cys Lys Arg Gly Arg Lys Gln Cys Lys Thr His Pro His Phe Val
100          105          110
Ile Pro Tyr Arg Cys Leu Val Gly Glu Phe Ile Ser Asp Ala Leu Leu

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	130					135					140						
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145					150					155					160		
Lys	Ser	Thr	Asn	Leu	His	Asp	Tyr	Gly	Met	Leu	Leu	Pro	Cys	Gly	Ile		
			165						170					175			
Asp	Lys	Phe	Arg	Gly	Val	Glu	Phe	Val	Cys	Cys	Pro	Leu	Ala	Glu	Glu		
			180					185					190				
Ser	Asp	Asn	Val	Asp	Ser	Ala	Asp	Ala	Glu	Glu	Asp	Asp	Ser	Asp	Val		
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Val	Val	Glu	Val	Ala	Glu	Glu	Glu	Glu	Val	Ala	Glu	Val	Glu	Glu	Glu		
225					230					235					240		
Glu	Ala	Asp	Asp	Asp	Glu	Asp	Asp	Glu	Asp	Gly	Asp	Glu	Val	Glu	Glu		
				245					250					255			
Glu	Ala	Glu	Glu	Pro	Tyr	Glu	Glu	Ala	Thr	Glu	Arg	Thr	Thr	Ser	Ile		
			260					265					270				
Ala	Thr	Thr	Thr	Thr	Thr	Thr	Thr	Glu	Ser	Val	Glu	Glu	Val	Val	Arg		
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Val	Pro	Thr	Thr	Ala	Ala	Ser	Thr	Pro	Asp	Ala	Val	Asp	Lys	Tyr	Leu		
	290					295					300						
Glu	Thr	Pro	Gly	Asp	Glu	Asn	Glu	His	Ala	His	Phe	Gln	Lys	Ala	Lys		
305					310					315					320		
Glu	Arg	Leu	Glu	Ala	Lys	His	Arg	Glu	Arg	Met	Ser	Gln	Val	Met	Arg		
				325					330					335			
Glu	Trp	Glu	Glu	Ala	Glu	Arg	Gln	Ala	Lys	Asn	Leu	Pro	Lys	Ala	Asp		
			340					345					350				
Lys	Lys	Ala	Val	Ile	Gln	His	Phe	Gln	Glu	Lys	Val	Glu	Ser	Leu	Glu		
		355					360					365					
Gln	Glu	Ala	Ala	Asn	Glu	Arg	Gln	Gln	Leu	Val	Glu	Thr	His	Met	Ala		
	370					375						380					
Arg	Val	Glu	Ala	Met	Leu	Asn	Asp	Arg	Arg	Arg	Leu	Ala	Leu	Glu	Asn		
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Tyr	Ile	Thr	Ala	Leu	Gln	Ala	Val	Pro	Pro	Arg	Pro	Arg	His	Val	Phe		
			405						410					415			
Asn	Met	Leu	Lys	Lys	Tyr	Val	Arg	Ala	Glu	Gln	Lys	Asp	Arg	Gln	His		
			420					425					430				
Thr	Leu	Lys	His	Phe	Glu	His	Val	Arg	Met	Val	Asp	Pro	Lys	Lys	Ala		
		435					440					445					
Ala	Gln	Ile	Arg	Ser	Gln	Val	Met	Thr	His	Leu	Arg	Val	Ile	Tyr	Glu		
	450					455					460						
Arg	Met	Asn	Gln	Ser	Leu	Ser	Leu	Leu	Tyr	Asn	Val	Pro	Ala	Val	Ala		
465					470												

610		615		620
Gly Ala Ile Ile Gly Leu Met Val Gly Gly Val Val Ile Ala Thr Val				
625		630		635
Ile Val Ile Thr Leu Val Met Leu Lys Lys Lys Leu Gly Thr Glu				
	645		650	655
Leu Gly Ser Thr Ser Pro Val Trp Trp Asn Ser Ala Asp Ile Leu Glu				
	660		665	670
Pro Val Asp Pro Arg Leu Glu Pro Trp Lys His Pro Gly Ser Gln Pro				
	675		680	685
Lys Thr Ala Cys Thr Asn Cys Tyr Cys Lys Lys Cys Cys Phe His Cys				
	690		695	700
Gln Val Cys Phe Met Thr Lys Ala Leu Gly Ile Ser Tyr Gly Arg Lys				
	705		710	715
Lys Arg Arg Gln Arg Arg Ala His Gln Asn Ser Gln Thr His Gln				
	725		730	735
Ala Ser Leu Ser Lys Gln Arg Ile Ser Ser Thr Val Ala Ala Ala Asp				
	740		745	750
Ala Ala Val Thr Pro Glu Glu Arg His Leu Ser Lys Met Gln Gln Asn				
	755		760	765
Gly Tyr Glu Asn Pro Thr Tyr Lys Phe Phe Glu Gln Met Gln Asn				
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&lt;211&gt; 2823

&lt;212&gt; DNA

&lt;213&gt; fusion protein - human

&lt;400&gt; 5

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&lt;210&gt; 6

&lt;211&gt; 941

&lt;212&gt; PRT

&lt;213&gt; fusion protein - human

&lt;400&gt; 6

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Gln Ile Ala Met Phe Cys Gly Arg Leu Asn Met His Met Asn Val Gln
 35          40          45
Asn Gly Lys Trp Asp Ser Asp Pro Ser Gly Thr Lys Thr Cys Ile Asp
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Thr Lys Glu Gly Ile Leu Gln Tyr Cys Gln Glu Val Tyr Pro Glu Leu
 65          70          75          80
Gln Ile Thr Asn Val Val Glu Ala Asn Gln Pro Val Thr Ile Gln Asn
 85          90          95
Trp Cys Lys Arg Gly Arg Lys Gln Cys Lys Thr His Pro His Phe Val
100          105          110
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145          150          155          160
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225          230          235          240
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Glu Thr Pro Gly Asp Glu Asn Glu His Ala His Phe Gln Lys Ala Lys
305          310          315          320

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Glu	Arg	Leu	Glu	Ala	Lys	His	Arg	Glu	Arg	Met	Ser	Gln	Val	Met	Arg
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Glu	Trp	Glu	Glu	Ala	Glu	Arg	Gln	Ala	Lys	Asn	Leu	Pro	Lys	Ala	Asp
			340					345					350		
Lys	Lys	Ala	Val	Ile	Gln	His	Phe	Gln	Glu	Lys	Val	Glu	Ser	Leu	Glu
		355					360					365			
Gln	Glu	Ala	Ala	Asn	Glu	Arg	Gln	Gln	Leu	Val	Glu	Thr	His	Met	Ala
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Arg	Val	Glu	Ala	Met	Leu	Asn	Asp	Arg	Arg	Arg	Leu	Ala	Leu	Glu	Asn
385					390				395					400	
Tyr	Ile	Thr	Ala	Leu	Gln	Ala	Val	Pro	Pro	Arg	Pro	Arg	His	Val	Phe
			405					410						415	
Asn	Met	Leu	Lys	Lys	Tyr	Val	Arg	Ala	Glu	Gln	Lys	Asp	Arg	Gln	His
		420						425					430		
Thr	Leu	Lys	His	Phe	Glu	His	Val	Arg	Met	Val	Asp	Pro	Lys	Lys	Ala
	435						440					445			
Ala	Gln	Ile	Arg	Ser	Gln	Val	Met	Thr	His	Leu	Arg	Val	Ile	Tyr	Glu
	450					455					460				
Arg	Met	Asn	Gln	Ser	Leu	Ser	Leu	Leu	Tyr	Asn	Val	Pro	Ala	Val	Ala
465					470				475						480
Glu	Glu	Ile	Gln	Asp	Glu	Val	Asp	Glu	Leu	Leu	Gln	Lys	Glu	Gln	Asn
			485					490						495	
Tyr	Ser	Asp	Asp	Val	Leu	Ala	Asn	Met	Ile	Ser	Glu	Pro	Arg	Ile	Ser
		500						505					510		
Tyr	Gly	Asn	Asp	Ala	Leu	Met	Pro	Ser	Leu	Thr	Glu	Thr	Lys	Thr	Thr
		515					520					525			
Val	Glu	Leu	Leu	Pro	Val	Asn	Gly	Glu	Phe	Ser	Leu	Asp	Asp	Leu	Gln
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Pro	Trp	His	Ser	Phe	Gly	Ala	Asp	Ser	Val	Pro	Ala	Asn	Thr	Glu	Asn
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Glu	Val	Glu	Pro	Val	Asp	Ala	Arg	Pro	Ala	Ala	Asp	Arg	Gly	Leu	Thr
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Thr	Arg	Pro	Gly	Ser	Gly	Leu	Thr	Asn	Ile	Lys	Thr	Glu	Glu	Ile	Ser
		580						585					590		
Glu	Val	Asn	Leu	Asp	Ala	Glu	Phe	Arg	His	Asp	Ser	Gly	Tyr	Glu	Val
		595					600					605			
His	His	Gln	Val	Leu	Val	Phe	Phe	Ala	Glu	Asp	Val	Gly	Ser	Asn	Lys
	610					615					620				
Gly	Ala	Ile	Ile	Gly	Leu	Met	Val	Gly	Gly	Val	Val	Ile	Ala	Thr	Val
625					630					635					640
Ile	Val	Ile	Thr	Leu	Val	Met	Leu	Lys	Lys	Lys	Lys	Leu	Gly	Thr	Glu
			645					650						655	
Leu	Gly	Ser	Thr	Ser	Pro	Val	Trp	Trp	Asn	Ser	Ala	Asp	Ile	Lys	Leu
		660						665					670		
Leu	Ser	Ser	Ile	Glu	Gln	Ala	Cys	Asp	Ile	Cys	Arg	Leu	Lys	Lys	Leu
		675					680					685			
Lys	Cys	Ser	Lys	Glu	Lys	Pro	Lys	Cys	Ala	Lys	Cys	Leu	Lys	Asn	Asn
	690					695					700				
Trp	Glu	Cys	Arg	Tyr	Ser	Pro	Lys	Thr	Lys	Arg	Ser	Pro	Leu	Thr	Arg
705					710					715					720
Ala	His	Leu	Thr	Glu	Val	Glu	Ser	Arg	Leu	Glu	Arg	Leu	Glu	Gln	Leu
			725						730					735	
Phe	Leu	Leu	Ile	Phe	Pro	Arg	Glu	Asp	Leu	Asp	Met	Ile	Leu	Lys	Met
		740						745					750		
Asp	Ser	Leu	Gln	Asp	Ile	Lys	Ala	Leu	Leu	Thr	Gly	Leu	Phe	Val	Gln
		755					760					765			
Asp	Asn	Val	Asn	Lys	Asp	Ala	Val	Thr	Asp	Arg	Leu	Ala	Ser	Val	Glu
	770					775					780				
Thr	Asp	Met	Pro	Leu	Thr	Leu	Arg	Gln	His	Arg	Ile	Ser	Ala	Thr	Ser
785					790					795					800
Ser	Ser	Glu	Glu	Ser	Ser	Asn	Lys	Gly	Gln	Arg	Gln	Leu	Thr	Val	Ser
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WO 03/072041

PCT/US03/05458  
PCT/US03/05458

Gly Ile Pro	Gly Asp Leu Ala <sup>4</sup> Pro	Pro Thr Asp	Val Ser Leu Gly Asp
	820	825	830
Glu Leu His	Leu Asp Gly Glu Asp	Val Ala Met	Ala His Ala Asp Ala
	835	840	845
Leu Asp Asp	Phe Asp Leu Asp	Met Leu Gly Asp	Gly Asp Ser Pro Gly
	850	855	860
Pro Gly Phe	Thr Pro His Asp	Ser Ala Pro Tyr	Gly Ala Leu Asp Met
	865	870	875
Ala Asp Phe	Glu Phe Glu Gln Met	Phe Thr Asp	Ala Leu Gly Ile Asp
	885	890	895
Glu Tyr Gly	Gly Asp Ile Gln His	Ser Gly Ala	Ala Ala Asp Ala Ala
	900	905	910
Val Thr Pro	Glu Glu Arg His	Leu Ser Lys Met	Gln Gln Asn Gly Tyr
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Glu Asn Pro	Thr Tyr Lys Phe	Phe Glu Gln Met	Gln Asn
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 <213> fusion protein - human

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WO 03/072041

PCT/US03/05458  
PCT/US03/05458

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tag

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&lt;210&gt; 8

&lt;211&gt; 941

&lt;212&gt; PRT

&lt;213&gt; fusion protein - human

&lt;400&gt; 8

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Gln Ile Ala Met Phe Cys Gly Arg Leu Asn Met His Met Asn Val Gln
35      40      45
Asn Gly Lys Trp Asp Ser Asp Pro Ser Gly Thr Lys Thr Cys Ile Asp
50      55      60
Thr Lys Glu Gly Ile Leu Gln Tyr Cys Gln Glu Val Tyr Pro Glu Leu
65      70      75      80
Gln Ile Thr Asn Val Val Glu Ala Asn Gln Pro Val Thr Ile Gln Asn
85      90      95
Trp Cys Lys Arg Gly Arg Lys Gln Cys Lys Thr His Pro His Phe Val
100     105     110
Ile Pro Tyr Arg Cys Leu Val Gly Glu Phe Ile Ser Asp Ala Leu Leu
115     120     125
Val Pro Asp Lys Cys Lys Phe Leu His Gln Glu Arg Met Asp Val Cys
130     135     140
Glu Thr His Leu His Trp His Thr Val Ala Lys Glu Thr Cys Ser Glu
145     150     155     160
Lys Ser Thr Asn Leu His Asp Tyr Gly Met Leu Leu Pro Cys Gly Ile
165     170     175
Asp Lys Phe Arg Gly Val Glu Phe Val Cys Cys Pro Leu Ala Glu Glu
180     185     190
Ser Asp Asn Val Asp Ser Ala Asp Ala Glu Glu Asp Asp Ser Asp Val
195     200     205
Trp Trp Gly Gly Ala Asp Thr Asp Tyr Ala Asp Gly Ser Glu Asp Lys
210     215     220
Val Val Glu Val Ala Glu Glu Glu Val Ala Glu Val Glu Glu Glu
225     230     235     240
Glu Ala Asp Asp Asp Glu Asp Asp Glu Asp Gly Asp Glu Val Glu Glu
245     250     255
Glu Ala Glu Glu Pro Tyr Glu Glu Ala Thr Glu Arg Thr Thr Ser Ile
260     265     270
Ala Thr Thr Thr Thr Thr Thr Thr Glu Ser Val Glu Glu Val Val Arg
275     280     285
Val Pro Thr Thr Ala Ala Ser Thr Pro Asp Ala Val Asp Lys Tyr Leu
290     295     300
Glu Thr Pro Gly Asp Glu Asn Glu His Ala His Phe Gln Lys Ala Lys
305     310     315     320
Glu Arg Leu Glu Ala Lys His Arg Glu Arg Met Ser Gln Val Met Arg
325     330     335
Glu Trp Glu Glu Ala Glu Arg Gln Ala Lys Asn Leu Pro Lys Ala Asp
340     345     350
Lys Lys Ala Val Ile Gln His Phe Gln Glu Lys Val Glu Ser Leu Glu

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WO 03/072041

PCT/US03/05458  
PCT/US03/05458

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 <211> 783

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&lt;213&gt; fusion protein - human

&lt;400&gt; 10

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      20          25          30
Gln Ile Ala Met Phe Cys Gly Arg Leu Asn Met His Met Asn Val Gln
      35          40          45
Asn Gly Lys Trp Asp Ser Asp Pro Ser Gly Thr Lys Thr Cys Ile Asp
      50          55          60
Thr Lys Glu Gly Ile Leu Gln Tyr Cys Gln Glu Val Tyr Pro Glu Leu
      65          70          75          80
Gln Ile Thr Asn Val Val Glu Ala Asn Gln Pro Val Thr Ile Gln Asn
      85          90          95
Trp Cys Lys Arg Gly Arg Lys Gln Cys Lys Thr His Pro His Phe Val
      100          105          110
Ile Pro Tyr Arg Cys Leu Val Gly Glu Phe Ile Ser Asp Ala Leu Leu
      115          120          125
Val Pro Asp Lys Cys Lys Phe Leu His Gln Glu Arg Met Asp Val Cys
      130          135          140
Glu Thr His Leu His Trp His Thr Val Ala Lys Glu Thr Cys Ser Glu
      145          150          155          160
Lys Ser Thr Asn Leu His Asp Tyr Gly Met Leu Leu Pro Cys Gly Ile
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Asp Lys Phe Arg Gly Val Glu Phe Val Cys Cys Pro Leu Ala Glu Glu
      180          185          190
Ser Asp Asn Val Asp Ser Ala Asp Ala Glu Glu Asp Asp Ser Asp Val
      195          200          205
Trp Trp Gly Gly Ala Asp Thr Asp Tyr Ala Asp Gly Ser Glu Asp Lys
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      225          230          235          240
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      260          265          270
Ala Thr Thr Thr Thr Thr Thr Thr Ser Val Glu Glu Val Val Arg
      275          280          285
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      290          295          300
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      305          310          315          320
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      325          330          335
Glu Trp Glu Glu Ala Glu Arg Gln Ala Lys Asn Leu Pro Lys Ala Asp
      340          345          350
Lys Lys Ala Val Ile Gln His Phe Gln Glu Lys Val Glu Ser Leu Glu
      355          360          365
Gln Glu Ala Ala Asn Glu Arg Gln Gln Leu Val Glu Thr His Met Ala
      370          375          380
Arg Val Glu Ala Met Leu Asn Asp Arg Arg Arg Leu Ala Leu Glu Asn
      385          390          395          400
Tyr Ile Thr Ala Leu Gln Ala Val Pro Pro Arg Pro Arg His Val Phe
      405          410          415
Asn Met Leu Lys Lys Tyr Val Arg Ala Glu Gln Lys Asp Arg Gln His
      420          425          430
Thr Leu Lys His Phe Glu His Val Arg Met Val Asp Pro Lys Lys Ala
      435          440          445
Ala Gln Ile Arg Ser Gln Val Met Thr His Leu Arg Val Ile Tyr Glu
      450          455          460

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 500 505 510  
 Tyr Gly Asn Asp Ala Leu Met Pro Ser Leu Thr Glu Thr Lys Thr Thr  
 515 520 525  
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 Pro Trp His Ser Phe Gly Ala Asp Ser Val Pro Ala Asn Thr Glu Asn  
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&lt;210&gt; 11

&lt;211&gt; 2352

&lt;212&gt; DNA

&lt;213&gt; fusion protein - human

&lt;400&gt; 11

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WO 03/072041

PCT/US03/05458  
PCT/US03/05458

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2823

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Thr Lys Glu Gly Ile Leu Gln Tyr Cys Gln Glu Val Tyr Pro Glu Leu
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Gln Ile Thr Asn Val Val Glu Ala Asn Gln Pro Val Thr Ile Gln Asn
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Trp Cys Lys Arg Gly Arg Lys Gln Cys Lys Thr His Pro His Phe Val
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WO 03/072041

PCT/US03/05458  
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WO 03/072041

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&lt;213&gt; fusion protein - human

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&lt;211&gt; 783

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&lt;213&gt; fusion protein - human

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&lt;212&gt; DNA

&lt;213&gt; fusion protein - human

&lt;400&gt; 29

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**WO 03/072041**

PCT/US03/05458

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WO 03/072041

PCT/US03/05458  
PCT/US03/05458

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&lt;400&gt; 31

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&lt;210&gt; 32

&lt;211&gt; 941

&lt;212&gt; PRT

&lt;213&gt; fusion protein - human

&lt;400&gt; 32

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WO 03/072041

PCT/US03/05458  
PCT/US03/05458

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WO 03/072041

PCT/US03/05458  
PCT/US03/05458

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WO 03/072041

PCT/US03/05458  
PCT/US03/05458

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 Gly Ser Asn Lys Gly Ala Ile Ile Gly Leu Met Val Gly Gly Val Val  
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WO 03/072041

PCT/US03/05458  
PCT/US03/05458

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WO 03/072041

PCT/US03/05458  
PCT/US03/05458

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(54) Title: ASSAYS TO MONITOR AMYLOID PRECURSOR PROTEIN PROCESSING

(57) Abstract: The present invention provides DNA constructs, genetically engineered host cells, and methods for identifying inhibitors of amyloid precursor protein (APP) processing. The methods provide for the convenient identification, in a single assay, of inhibitors of  $\beta$ -secretase and  $\gamma$ -secretase as well as other forms of APP processing. The methods rely on fusion proteins of APP and transcription factors in which APP processing releases the transcription factors, allowing the transcription factors to activate transcription of a reporter gene. Inhibitors are identified as substances that block or diminish transcription factor release from the fusion protein, thereby causing a diminution of reporter gene readout.

WO 2003/072041 A3

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US03/05458

**A. CLASSIFICATION OF SUBJECT MATTER**

IPC: C07H 21/02( 2006.01);C12P 21/06( 2006.01);C12N 1/20( 2006.01),15/00( 2006.01)

USPC: 536/23.1;435/69.1,252.3,320.1

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 536/23.1; 435/69.1, 252.3, 320.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5,811,633 A (Haseltine et al) 1 September 1998 (01.09.1998), nucleic acid encoding HIV-1Tat transcription factor and trans-activating activity.	1-16
A	US 5,877,015 A (Hardy et al) 2 March 1999 (02.03.1999), SEQ ID NO:4 discloses APP695.	1-16
A	US 5,811,063 (Wadsworth et al) 22 September 1998 (22.09.1998), see claims disclosure of APP695.	1-16

☐ Further documents are listed in the continuation of Box C.☐ See patent family annex.

Special categories of cited documents:	
"A" document defining the general state of the art which is not considered to be of particular relevance	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"E" earlier application or patent published on or after the international filing date	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O" document referring to an oral disclosure, use, exhibition or other means	"&" document member of the same patent family
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

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Date of mailing of the international search report

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Commissioner of Patents  
P.O. Box 1450  
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Authorized officer

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Telephone No. 308-1235

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# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US03/05458

## Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claim Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claim Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claim Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:  
Please See Continuation Sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1-16

Remark on Protest

☐  
☐

The additional search fees were accompanied by the applicant's protest.

No protest accompanied the payment of additional search fees.



## INTERNATIONAL SEARCH REPORT

PCT/US03/05458

### BOX II. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group 1 Claim(s) 1-16, drawn to a DNA molecule and method of identifying a substance that inhibits APP processing.

Group 2 Claim(s) 17-20, drawn to a method of identifying a substance that inhibits APP comprising a transcription factor fused to APP.

This application contains claims directed to more than one species of the generic invention. These species are deemed to lack unity of invention because they are not so linked as to form a single general inventive concept under PCT Rule 13.1. The species listed do not share a common structure or function.

The species are as follows:

The following claim(s) are generic:

Group 1, Claims 1, 4, 5, 6, 7, 13, 14, 15, 16, all APP species listed therein.

In order for more than one species to be examined, the appropriate additional examination fees must be paid.

Applicant is invited to select one species per generic claim listed per group elected. The inventions listed as Groups 1-2 do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Group 1 is drawn to the special technical feature of a DNA molecule comprising APP695 fused to a transcription factor, which is not required by any of the other groups.

Group 2 is drawn to the special technical feature of a fusion protein comprising APP which is cleaved by both beta-secretase and gamma-secretase, which is not required by any of the other groups.

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